Antitumor Effects and Pharmacokinetics of Aclacinomycin A Carried by Injectable Emulsions Composed of Vitamin E, Cholesterol, and PEG-Lipid

JUNPING WANG, YOSHIE MAITANI, KOZO TAKAYAMA

Department of Pharmaceutics, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan

Received 11 June 2001; revised 9 November 2001; accepted 18 December 2001

Published online 12 March 2002 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/fos.10104

ABSTRACT: The aim of this study was to prepare injectable emulsions of aclacinomycin A (E-ACM) and evaluate its acute toxicity, antitumor effects, and pharmacokinetics. In E-ACM, the surfactants were polyethylene glycol-lipid and cholesterol, and the oil phase was a vitamin E solution of ACM. The particle size distribution and the zeta potential of E-ACM were measured by the laser light dynamic scattering method. The ACM-loading efficiency was measured by using Sephadex G50 column chromatography. The acute toxicity, antitumor effects, and pharmacokinetics of E-ACM were studied in C57BL/6 mice bearing mouse murine histiocytoma M5076 tumors. The average diameter, zeta potential, and ACM-loading efficiency of E-ACM were 123.0 ± 1.2 nm, -12.67 ± 1.35 mV, and 96.3 ± 0.3% (n = 3), respectively. When stored at 7°C in the dark for 1 year, the average diameter and ACM-loading efficiency of E-ACM changed into 126.3 ± 2.3 nm and 97.4 ± 0.8%, respectively, whereas 6.5 ± 0.2% ACM decomposition was observed (n = 3). The plasma areas under the biodistribution curves (AUC)₀⁻₄₈h of E-ACM was significantly greater than that of free ACM (F-ACM). The heart, lung, and kidney AUC₀⁻₄₈h of E-ACM were significantly smaller than those of F-ACM whereas the liver and spleen AUC₀⁻₄₈h of E-ACM were not significantly different from those of F-ACM. The tumor AUC₀⁻₄₈h of E-ACM was significantly greater than that of F-ACM. E-ACM had lower acute toxicity and greater potential antitumor effects than F-ACM in M5076 tumor-bearing C57BL/6 mice. E-ACM is a useful tumor-targeting drug delivery system.


Keywords: emulsions; aclacinomycin A; pharmacokinetics; acute toxicity; antitumor effects

INTRODUCTION

Aclacinomycin A (ACM) hydrochloride is an antitumor antibiotic isolated from Streptomyces galilaeus. The drug exerts its antitumor activity by acting as a catalytic inhibitor of topoisomerase II and as a topoisomerase I poison. ACM is widely used for the treatment of carcinoma of the stomach, pulmonary carcinoma, oophoroma, malignant lymphadenoma, and acute leukemia. The major problems associated with ACM cancer chemotherapy are its toxic actions, among which cardiomyopathy, and arrest of bone marrow are the most serious. To reduce the toxic responses and increase the antitumor effect of ACM, conventional liposomes, nanoparticles, microspheres, lipiodol, and activated carbon particles have been used as carriers of ACM. After intravenous (iv) injection, the majority of conventional liposomes or nanoparticles of ACM are taken up by the mononuclear phagocytic system (MPS), which means they are MPS-targeting drug delivery systems (DDS) instead of tumor-targeting DDS.
Microspheres, lipiodol, and activated carbon particles of ACM are administered by local injection to target ACM to the tumor tissue or hepatic artery catheter perfusion for treatment of liver cancer.\(^7\)\(^\text{-}\)\(^9\) These methods have been confirmed to be effective but are not applicable for the systemic treatment of cancer. To overcome these problems, it is necessary to develop an injectable tumor-targeting DDS as a carrier of ACM.

Over the past 10 years, significant advances have been made in injectable tumor-targeting DDS, of which sterically stabilized liposomes (stealth liposomes) and long-circulating emulsions are the most potentially useful. Stealth liposomes containing PEG-lipid (a polyethylene glycol-derivatized phospholipid) have been reported to circulate in the bloodstream of mice for several days. Finally, the stealth liposomes accumulate in the tumor tissue because of the higher permeability of the blood vessels of the tumor tissue. However, the procedure for preparation of stealth liposomes is very complicated.\(^10\) In addition, ACM is a lipophilic agent and is not stable in water.\(^11\) Therefore, long circulating emulsions may be more suitable for delivery of ACM. In recent years, considerable emphasis has been given to the development of long-circulating emulsions used as carriers of lipophilic drugs. It was reported that inclusion of amphipathic PEG as an emulsifier into oil-in-water emulsions was a very effective method to prolong the blood half-life of the emulsions.\(^12\) Long-circulating emulsions have been used as carriers of paclitaxel, and paclitaxel emulsions demonstrated good antitumor activity in vitro when compared with commercial paclitaxel.\(^13\) No in vivo studies were reported. Recently, a filter-sterilizable emulsion of paclitaxel containing PEG-lipid was reported to have lower toxicity and higher antitumor efficacy than the commercial drug.\(^14\) We designed an injectable emulsion composed of PEG-lipid, cholesterol, and vitamin E as a carrier of ACM (E-ACM).

The aim of this study was to characterize E-ACM and evaluate its acute toxicity, antitumor effects, and pharmacokinetics.

**Experimental Section**

**Chemicals**

ACM hydrochloride for injection (Aclacinon\(^\circ\)) was kindly supplied by Yamanouchi Pharm. Co., Ltd. (Tokyo, Japan). PEG-lipid (polyethylene glycol derivative of distearoylphosphatidyl ethanolamine, PEG-DSPE, mean molecular weight of PEG: 2000) was kindly supplied by NOF Co. Ltd. (Tokyo, Japan). (±)-α-Tocopherol (vitamin E) was obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Cholesterol was purchased from Tokyo Chemistry Industry Co. (Tokyo, Japan). Chemicals for high-pressure liquid chromatography (HPLC) were of HPLC grade and all other chemicals were of analytical grade.

**Animal and Tumor Cells**

The animals used were male C57BL/6 mice, weighing 18–20 g, supplied by Tokyo Animal Experiment Center (Tokyo, Japan). All mice used in the study were raised in the SPF animal laboratory. Mouse murine histiocytoma M5076 tumor cells were supplied by the Cancer Chemotherapy Center of the Japanese Foundation for Cancer Research (Tokyo, Japan). After four transplant generations, the tumor cells were used in this study.

**Preparation of E-ACM**

ACM hydrochloride, vitamin E, cholesterol, and PEG-DSPE (1:3:3:3, w/w/w) were dissolved in an appropriate volume of ethanol. The ethanol was removed using an evaporator under N\(_2\) gas and finally a semisolid solution was formed. An appropriate volume of 0.9% NaCl aqueous solution was added to the semisolid solution and emulsions formed instantly. To obtain uniform particle size distribution, the mixture was homogenized using a bio-mixer (Nissei Co., Tokyo, Japan) at 2.3 x 10\(^4\) rpm twice for 6 min each time in ice water and then sterilized by filtration three times through a sterile syringe-driven filter unit with a pore size of 100 nm (Millipore Co., Bedford, MA). Finally, the emulsions were injected into ampules (10 mL/ampule), which were sealed after oxygen was driven out with aseptic N\(_2\) gas. The whole procedure was performed on a clean bench and all materials were pyrogen-free and sterilized. Each ampule contained 15 mg of vitamin E, 15 mg of PEG-DSPE, 15 mg of cholesterol, and 5 mg of ACM hydrochloride in 10 mL of saline. The emulsions without ACM were used as vehicle controls in the subsequent experiments to determine antitumor effects. Free-ACM (F-ACM) was ACM saline solution (0.5 mg/mL).

**JOURNAL OF PHARMACEUTICAL SCIENCES, VOL. 91, NO. 4, APRIL 2002**
Characterization of E-ACM

The particle size distribution and the zeta potential of three batches of E-ACM were determined using a laser light scattering instrument (ELS800; Otsuka Electronics, Osaka, Japan) by the dynamic light scattering method and the electrophoresis light scattering method, respectively. The ACM-loading efficiency of E-ACM was taken as the percentage of ACM carried by the emulsions and was determined by Sephadex G50 column chromatography. E-ACM was separated from F-ACM in the Sephadex G50 column using a mobile phase of 0.9% NaCl aqueous solution. The amounts of ACM in the free fraction and the E-ACM fraction were determined by HPLC as described below in the Pharmacokinetics section. The ACM-loading efficiency was calculated according to the following equation:

\[
\text{ACM-loading efficiency (\%) = } (1 - A_f/A_t) \times 100
\]

where \( A_f \) was the amount of F-ACM and \( A_t \) was the total amount of ACM.

The stability of E-ACM was monitored by particle size, ACM-loading efficiency, and ACM content changes of three batches of E-ACM stored at 7°C in the dark for 1 year.

Acute Toxicity and Antitumor Effects

To evaluate the acute toxicity of E-ACM, the mice were inoculated intraperitoneally with \( 1 \times 10^6 \) M5076 tumor cells per mouse and divided into groups of six animals per group. The tumor cells were removed from normal donor mice under anesthesia with methoxyflurane inhalation. One day after inoculation, the two groups of mice were administered F-ACM and E-ACM by iv injection slowly (in 15 min) at a dose of 24 mg ACM kg\(^{-1}\) animal.

To evaluate the antitumor effects of E-ACM, mice were inoculated subcutaneously with \( 1 \times 10^6 \) M5076 tumor cells and divided into groups of 12 animals per group (day 0). Treatment started on day 5 after inoculation. Animals were treated with the emulsions without ACM (vehicle control), F-ACM, or E-ACM by iv injection three times with an interval of 5 days after inoculation of the M5076 tumor cells. Two doses were established for F-ACM and E-ACM, 2.5 and 5.0 mg ACM kg\(^{-1}\), respectively. The corresponding doses of the vehicle controls were 5 and 10 mL kg\(^{-1}\). Mice in the blank control group were not treated. The mice were killed on day 18 and the antitumor effect was reflected in the tumor growth suppression rate \((T/C)\) calculated by the following equation: \(T/C = (W_c - W_t)/W_c \times 100\%\), where \(W_c\) and \(W_t\) are the mean tumor weights (grams) of the blank control and the treated mice, respectively. The results were analyzed statistically using the Bonferroni/Dunn post hoc test. When comparisons between groups yielded a value for \(p < 0.05\), the difference between those groups was considered statistically significant.

Pharmacokinetics

C57BL/6 mice (male, 18–20 g) were selected to evaluate the pharmacokinetics of E-ACM and were inoculated subcutaneously with M5076 tumor cells. Two weeks later, the mice were fasted for 12 h and given only water. Three mice were used at each time point. Both E-ACM and F-ACM were administered by iv injection at a single dose of 10 mg ACM kg\(^{-1}\). The samples (blood, tumor, heart, liver, spleen, lung, and kidney) were obtained at 1/30, 7/60, 1/3, 2/3, 2, 4, 8, 24, and 48 h after iv injection. The blood was immediately centrifuged at 3000 rpm for 10 min. The plasma was separated and kept at −20°C with the other samples until analysis. ACM was extracted from the biological samples and determined as described previously. The HPLC system was composed of an LC-10AS pump (Shimadzu Co., Ltd., Kyoto, Japan), an SII-10A autoinjector (Shimadzu Co., Ltd., Japan), an RF-10AXL fluorescence detector (EX = 435 nm, EM = 505 nm; Shimadzu Co., Ltd., Japan) and a μBondapak\textsuperscript{TM} C18 column (3.9 × 300 mm; Waters, Milford, USA). The mobile phase was acetonitrile/methanol/water = 40:90:70 (v/v/v, pH = 3.76, adjusted with 0.1 M H\textsubscript{3}PO\textsubscript{4}) and the flow rate was 1.0 mL/min. The concentration of ACM in each sample was determined using a calibration curve, using doxorubicin as the internal standard. Data were analyzed using the nonlinear least-squares fitting program, in which AIC (Akaike's information criterion) was used to determine an appropriate model to fit the plasma ACM concentration data. The areas under the biodistribution curves (from 0.03 to 48 h) \((\text{AUC}_{0.03-48h})\) of E-ACM and F-ACM were calculated using the trapezoid method. The results were analyzed statistically using the Welch t test and expressed as one-way \(p\) value. When comparisons between groups yielded a value for \(p < 0.05\), the difference between those groups was considered statistically significant.
RESULTS

Characterization of E-ACM

The particle size distribution of E-ACM was 80–150 nm and the average diameter was 123.0 ± 1.2 nm (n = 3). The zeta potential and ACM-loading efficiency of three batches of E-ACM were -12.67 ± 1.35 mV and 96.3 ± 0.3% (n = 3), respectively. When stored at 7°C in the dark for 1 year, the average diameter and ACM-loading efficiency of E-ACM changed into 126.3 ± 2.3 nm and 97.4 ± 0.8% (n = 3), respectively, whereas 6.5 ± 0.2% ACM decomposition was observed (n = 3).

Table 1. Comparison of the Antitumor Effects of E-ACM and F-ACM in the Solid M5076 Tumor Model

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Dose (mg ACM kg⁻¹)</th>
<th>MTW ± SD (g)</th>
<th>T/C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Control²</td>
<td>1.59 ± 0.33</td>
<td>—</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Control¹</td>
<td>1.49 ± 0.29</td>
<td>6</td>
</tr>
<tr>
<td>F-ACM</td>
<td>2.5</td>
<td>1.04 ± 0.18</td>
<td>35</td>
</tr>
<tr>
<td>E-ACM</td>
<td>2.5</td>
<td>0.93 ± 0.28</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.90 ± 0.21</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.33 ± 0.15</td>
<td>79</td>
</tr>
</tbody>
</table>

¹Administered by iv injection three times at intervals of 5 days (treatment started on day 5 after inoculation of M5076 tumor cells).
²Mean tumor weight of 12 mice.
³T/C (%) = (MTWc - MTWt)/MTWc, where MTWc and MTWt are the mean tumor weights of the blank control and the treated mice, respectively.
⁴Untreated.
⁵Vehicle: 5 mL kg⁻¹.
⁶Vehicle: 10 mL kg⁻¹.
⁷p > 0.05, compared with blank control (untreated).
⁸p > 0.05, compared with that of F-ACM at the dose of 2.5 mg ACM kg⁻¹.
⁹p < 0.01, compared with F-ACM at the dose of 5.0 mg ACM kg⁻¹.

Acute Toxicity and Antitumor Effects

A comparison of the acute toxicity of F-ACM and E-ACM is shown in Figure 1. All mice in the F-ACM group died within 10 days, and those in the E-ACM group died within 18–21 days. The life span of the mice in the control group was 17–21 days. Therefore, E-ACM had lower acute toxicity than F-ACM in M5076 tumor-bearing C57BL/6 mice. The antitumor effects of E-ACM are presented in Table 1. At the dose of 2.5 mg ACM kg⁻¹, the T/C value of E-ACM groups was not significantly higher than that of F-ACM. However, when the dose was increased to 5.0 mg ACM kg⁻¹, the T/C of the E-ACM group was 79%, significantly higher than that of the F-ACM group (43%).

Pharmacokinetics

Plasma clearance and biodistribution curves of F-ACM and E-ACM are shown in Figure 2 and the AUC₀⁻⁴₈h values of E-ACM and F-ACM are shown in Table 2. The plasma concentration–time curve of E-ACM was best fitted to a triexponential decay curve and the pharmacokinetic parameters are presented in Table 3. Unlike E-ACM, after iv injection, F-ACM left the blood circulation system immediately and was perhaps taken up by the non-blood tissues, and about 1 h later, the F-ACM taken up by the tissues seemed to be released into the blood (Fig. 2A). The plasma AUC₀⁻⁴₈h of E-ACM was significantly greater than that of F-ACM (Table 2). The heart, lung, and kidney AUC₀⁻⁴₈h of E-ACM were significantly smaller than those of F-ACM whereas the liver and spleen AUC₀⁻⁴₈h of E-ACM were not significantly different from those of F-ACM (Table 2). In addition, the tumor AUC₀⁻⁴₈h of E-ACM was significantly greater than that of F-ACM. Therefore, E-ACM is a tumor-targeting DDS.
Figure 2. Plasma concentration–time curves and biodistribution comparison of injectable microemulsions of aclacinomycin A (E-ACM) and free aclacinomycin A (F-ACM) after iv injection at a dose of 10 mg ACM kg⁻¹ in C57BL/6 mice bearing solid M5076 tumors. (A) Plasma, and the smooth curve was the triexponential decay curve of E-ACM; (B) heart; (C) liver; (D) kidney; (E) spleen; (F) lung; (G) tumor. Sampling times were 1/30, 7/60, 1/3, 2/3, 2, 4, 8, 24, and 48 h. ●, E-ACM; △, F-ACM. Results are given as means ± SD of three mice.
**Table 2.** AUC<sub>0.03-48</sub> h Comparison of the Plasma Drug Concentration–time and Biodistribution Curves of F-ACM and E-ACM<sup>a</sup>

<table>
<thead>
<tr>
<th>Tissues</th>
<th>F-ACM</th>
<th>E-ACM</th>
<th>p Value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>21.4 ± 4.3</td>
<td>54.3 ± 10.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart</td>
<td>799.9 ± 152.0</td>
<td>447.1 ± 67.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>597.9 ± 113.6</td>
<td>497.7 ± 69.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Spleen</td>
<td>973.8 ± 282.4</td>
<td>871.9 ± 218.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Lung</td>
<td>331.3 ± 102.7</td>
<td>154.9 ± 44.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Kidney</td>
<td>638.3 ± 248.8</td>
<td>379.3 ± 49.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tumor</td>
<td>94.4 ± 12.3</td>
<td>212.0 ± 65.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are presented as mean ± SE (n = 3).

<sup>b</sup>AUC<sub>0.03-48</sub> of E-ACM was compared with that of F-ACM in corresponding tissue.

**DISCUSSION**

Unlike the long circulating emulsions reported previously,<sup>12,13</sup> E-ACM formed spontaneously. In addition, E-ACM could be sterilized by simply passing emulsions through a sterile syringe-driven filter unit with a pore size of 100 nm by hand. According to the theory of emulsion, macroemulsion formation generally requires vigorous mixing, whereas microemulsions tend to form spontaneously. Therefore, E-ACM seems to be a microemulsion (thermodynamically stable emulsion < 150 nm in size) and can be prepared easily.

When emulsions are administered by iv injection, they are rapidly taken up by the MPS in the liver and spleen.<sup>17,18</sup> This is advantageous to target drugs to the MPS, but is an obstacle for delivery of drugs to targets outside the MPS. Unlike the emulsions described above, the liver and spleen AUC<sub>0.03-48</sub> of E-ACM were not increased compared with those of F-ACM (Table 2), which may be attributed to the steric barrier of the surface-grafted PEG chains of E-ACM. In addition, E-ACM (80–150 nm) does not efficiently cross the endothelial cell barrier present in most normal tissues because the diameter of the pores of the capillaries in the normal tissues is generally <80 nm.<sup>16</sup> Therefore, after iv injection of E-ACM, less ACM was taken up by the heart, lung, or kidney tissues as compared with F-ACM. This was supported by comparison of the biodistribution curves of E-ACM and F-ACM. Therefore, when administered in the E-ACM formulation, the drug circulated in the bloodstream for a longer time compared with F-ACM, which seemed to be mainly due to the reduced biodistribution of E-ACM to the normal tissues outside the MPS. The total plasma and blood volumes of mice are approximately 0.49 L kg<sup>-1</sup> and 0.75 L kg<sup>-1</sup>, respectively.<sup>20</sup> The V<sub>1</sub> of E-ACM was 0.4 L kg<sup>-1</sup>, which was close to the total mouse plasma volume, indicating that E-ACM was present mainly in the plasma in the V<sub>1</sub> compartment. The V<sub>2</sub> of E-ACM was 0.7 L kg<sup>-1</sup>, close to the mouse blood volume, indicating that ACM of E-ACM was present mainly in the bloodstream in the V<sub>2</sub> compartment. The V<sub>3</sub> of E-ACM was 19.1-fold greater than the total mouse blood volume, suggesting that E-ACM left the bloodstream and was taken up by other tissues. However, to confirm these inferences, further work is needed to determine E-ACM in plasma directly. Unlike E-ACM, after iv injection, F-ACM left the blood circulation system immediately and was perhaps taken up by the non-blood tissues. About 1 hour later, the F-ACM taken up by the tissues seemed to be released into the blood (Fig. 2A), which was different from that of ACM in rabbits and dogs reported previously.<sup>15</sup> This difference might have been the result of the species variation and the different sampling time schedules.

Unlike the normal tissues in living systems, the tumor vasculature has been shown to be relatively leaky and less permeoselective than normal vessels.<sup>21,22,23</sup> The vascular permeability in the tumor tissue is probably governed by diffusion across the vessel wall that allows the penetration of particles up to 400 nm in diameter. The particle size distribution of E-ACM is <150 nm, which made it possible for long circulating E-ACM to accumulate in tumor

**Table 3.** Pharmacokinetic Parameters<sup>a</sup> of E-ACM<sup>b</sup>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (1 h&lt;sup&gt;-1&lt;/sup&gt; kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.09 (0.05)</td>
</tr>
<tr>
<td>Vss (1 kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>15.41 (5.14)</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2α&lt;/sub&gt; (h)</td>
<td>0.18 (0.08)</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2β&lt;/sub&gt; (h)</td>
<td>1.62 (0.67)</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2γ&lt;/sub&gt; (h)</td>
<td>99.00 (0.46)</td>
</tr>
<tr>
<td>V&lt;sub&gt;1&lt;/sub&gt; (1 kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.42 (0.14)</td>
</tr>
<tr>
<td>V&lt;sub&gt;2&lt;/sub&gt; (1 kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.65 (0.18)</td>
</tr>
<tr>
<td>V&lt;sub&gt;3&lt;/sub&gt; (1 kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>14.34 (7.31)</td>
</tr>
</tbody>
</table>

<sup>a</sup>The following parameters were included: CL, systemic clearance; Vss, distribution volume at steady state; T<sub>1/2α</sub>, T<sub>1/2β</sub>, and T<sub>1/2γ</sub>, half-lives of α, β, and γ phases; V<sub>1</sub>, V<sub>2</sub>, and V<sub>3</sub>, apparent distribution volumes of α, β, and γ phases.

<sup>b</sup>Values in parentheses represent standard error (n = 3).
tissues. Therefore, the tumor AUC₀.₀₃₋₄₈₉ of E-ACM was significantly greater than that of F-ACM (Table 2), and thus E-ACM was a tumor-targeting DDS. The heart AUC₀.₀₃₋₄₈₉ of E-ACM was significantly smaller than that of F-ACM (Table 2). The cardiac toxicity was one of the most serious toxic actions of ACM, and therefore the acute toxicity of E-ACM was much lower than that of F-ACM (Fig. 1). E-ACM is a tumor-targeting DDS, and therefore the antitumor effect of E-ACM was significantly greater than that of F-ACM (Table 1).

In conclusion, E-ACM has lower toxicity and higher antitumor effect than F-ACM. E-ACM is a useful tumor-targeting DDS.

ACKNOWLEDGMENTS

This work was supported by grants from the Japan Society for Promotion of Science and the Ministry of Education, Science, Sports, and Culture, Japan.

REFERENCES