[Chem. Pharm. Bull. 33(7)2968—2973(1985)]

Blood Dispositions of Mitomycin C and a Lipophilic Prodrug after Intramuscular and Intravenous Administration in Liposomes and O/W Emulsion

HITOSHI SASAKI,[a] TOSHIYUKI KAKUTANI,[b] MITSURU HASHIDA,[b] TOSHIKIRO KIMURA,[c] and HITOSHI SEZAKI*,[b]

Faculty of Pharmaceutical Sciences, Nagasaki University,[a] Bunkyomachi 1–14, Nagasaki 852, Japan, Faculty of Pharmaceutical Sciences, Kyoto University,[b] Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto 606, Japan, and Faculty of Pharmaceutical Sciences, Okayama University,[c] Tsushimanaka 1–1–1, Okayama 700, Japan

(Received October 12, 1984)

The potential utility of lipidic dosage forms incorporating a lipophilic prodrug of mitomycin C (MMC), nonyloxycarbonyl MMC, was evaluated by determining blood levels after intramuscular and intravenous administration of different formulations. After intramuscular injection of MMC in the forms of liposomes, O/W emulsion and dimethyl sulfoxide (DMSO) solution, MMC appeared in the circulation at an early stage and disappeared rapidly regardless of dosage form. However, neither MMC nor prodrug could be detected in the blood after injection of the prodrug in lipidic dosage forms. These results suggest that formulations consisting of prodrug and lipidic carriers would show less severe systemic side effects than formulations of MMC for local injection. In the same experiment, the lipid component of liposomes was detected in the blood, but that of emulsion did not appear in the blood. On the other hand, although intravenous injection of the prodrug in DMSO solution resulted in rapid conversion to MMC, slow conversion was observed when this prodrug was administered in the form of O/W emulsion. These results suggest that the prodrug was retained in these carriers and released gradually, followed by subsequent rapid conversion to MMC in the plasma. Thus, wide potential applicability of these systems for controlling the fate of MMC was demonstrated.

Keywords——anticancer agent; mitomycin C; lipophilic prodrug; O/W emulsion; liposome; dosage form; blood level; urinary excretion; sustained release; drug delivery system

Recently, the use of lipid dispersion systems as carriers of antitumor drugs has been extensively developed and has attracted special interest in cancer chemotherapy.[1] A number of chemotherapeutic agents have been encapsulated in lipidic carriers such as liposomes and O/W emulsion and the usefulness of these preparations has been demonstrated in systemic and local administration.[2] However, there is a drawback to the encapsulation of certain drugs to these lipid dispersion systems; *i.e.*, amphiphilic compounds (most antitumor agents) are poorly entrapped in these carrier systems.[3] Although numerous attempts have been reported to improve the entrapment efficiency by ordinary pharmaceutical approaches,[4] no major improvement has been accomplished.

The ability to incorporate drugs successfully into liposomes and O/W emulsions depends not only on the characteristics of the lipid dispersion system but also on the physicochemical properties of the drug. Therefore, in a previous study,[5] we designed a lipidic formulation of an amphiphilic antitumor antibiotic, mitomycin C (MMC), by utilizing chemical transformation to prodrugs with adequate physicochemical properties for incorporation in the lipidic carriers, and their usefulness as lymphotropic delivery systems for preventing lymphatic metastasis of cancer in local application was demonstrated. In addition to the enhancement of lymphatic delivery, the local administration of these formulations may lead to a sustained

plasma level and decreased systemic side effects such as myelosuppression.[6] Furthermore, in the case of intravenous injection, encapsulation of the drug in the lipid dispersion systems may prolong the life time of drug in the circulation.[7]

Therefore, in order to evaluate the therapeutic usefulness of lipidic delivery systems combined with a chemical modification approach, the disposition of MMC and its prodrug in the blood after intramuscular and intravenous injection of lipidic forms was investigated in the present study.

Experimental

Materials——MMC was supplied by Kyowa Hakko Kogyo Co.; partition coefficient between water and *n*-octanol, 0.41; solubility in water, 2.73 mM, and sesame oil, 0.0018 mM; entrapment percent in liposomes, 0.1% and O/W emulsion, 1.0%. The lipophilic derivative, nonyloxycarbonyl MMC, was synthesized by reported method[8]; partition coefficient between water and *n*-octanol, 3637; solubility in water, 0.0003 mM, and sesame oil, 15.17 mM; entrapment percent in liposomes, 99.9%, and O/W emulsion, 99.9%. Egg phosphatidylcholine was prepared from egg yolks by an ordinary method.[9] Nonionic surfactant, the polyoxyethylene derivative of hydrogenated castor oil (HCO-60), was obtained from Nikko Chemicals Co. Labeled lipids, [^{14}C]palmitoyl phosphatidylcholine and [^{14}C]tripalmitic glycerol, were purchased from Japan Radioisotope Association. All other chemicals were of reagent grade and were obtained commercially.

Preparation of Liposomes and O/W Emulsion——Liposomes were prepared by the method of Bangham and Horne.[10] Phosphatidylcholine and drug at a molar ratio of 4 to 1 were dissolved in chloroform and the solution was evaporated under a vacuum to give a thin lipid film. The dry lipid film was then suspended in a saline solution by Vortex mixing, and the resulting suspension was sonically disrupted at 0 °C for 3 min under a nitrogen atmosphere.

O/W emulsion was prepared with 4 volumes of sesame oil containing drugs and 6 volumes of aqueous phase containing a surfactant (7.5% (v/v) HCO-60). Emulsification was carried out by sonication.

Finally, both formulations and 40% DMSO–saline solution were prepared to have drug concentration of 5.98 and 4.6 mM for intramuscular and intravenous experiments, respectively.

Animal Intramuscular Injection Experiment——Intramuscular injection was carried out as described in the previous report.[5] Male Wistar albino rats weighing between 200 and 220 g were injected with 3 μmol/kg in various formulations into the right thigh muscle. At various times after injection, rats were sacrificed and the blood was removed *via* the aorta. Nonyloxycarbonyl MMC was extracted from an aliquot of the blood with ethyl acetate and assayed by high-performance liquid chromatography (HPLC). Another aliquot was washed with chloroform and the aqueous phase was used for bioassay of MMC.

Radioisotope Experiment——Lipids incorporating [^{14}C]palmitoyl phosphatidylcholine and [^{14}C]tripalmitic glycerol were used. The procedures for the preparation of dosage forms containing drugs and for the animal experiment were the same as those used in the "cold" experiments. The lipids of samples were detected by radioactivity assay.

Animal Intravenous Injection Experiment——Male Wistar albino rats weighing between 180 and 220 g were anesthesized with pentobarbital and drugs were injected in various formulations into the femoral vein at the dose of 15 μmol/kg. At various times after injection, blood samples were taken from the jugular vein and urine samples were obtained by bladder canulation. The blood samples and urine samples were diluted if necessary and analyzed by bioassay and HPLC for MMC and its prodrug in accordance with the procedures described for the intramuscular injection experiments.

Bioassay and HPLC Assay——Bioassay of MMC was carried out by an ordinary paper disc method using *Escherichia coli* B as a test organism.

Nonyloxycarbonyl MMC was determined by HPLC (TRIROTAR, Jasco) with detection at 350 nm. The stationary phase was a Cosmosil 5C_{18} packed column (4.6 × 150 mm, Nakarai Chemicals), and 80% methanol in water was used as the mobile phase at a flow rate of 0.8 ml/min.

Radioactivity Assay——The blood samples were digested with 1 N NaOH and decolorized with 100 μl of *tert*-butylhydroperoxide (BHP). Samples were analyzed for ^{14}C in a Packard model 2425 scintillation counter. A carbon-spillover curve was constructed for each tissue under investigation to correct for ^{14}C in the tritium channel. Counting efficiency was estimated by external standardization. All samples were counted to achieve a counting error of 5% or less.

Results

Incorporation of Drugs into Lipidic Dosage Forms

The lipophilic prodrug of MMC, nonyloxycarbonyl MMC, was used with the lipid

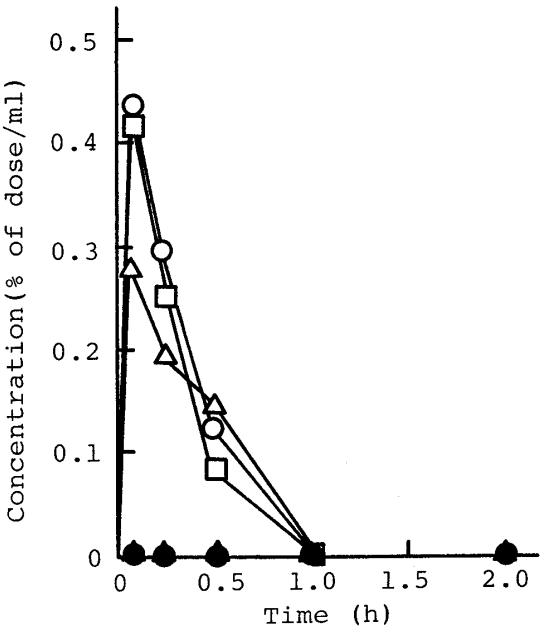

Fig. 1. Blood Concentration of MMC or Nonyloxycarbonyl MMC after Intramuscular Administration in Liposomes and O/W Emulsion

□, MMC in saline; ○, MMC in liposomes; △, MMC in O/W emulsion; ●, nonyloxycarbonyl MMC in liposome; ▲, nonyloxycarbonyl MMC in O/W emulsion.

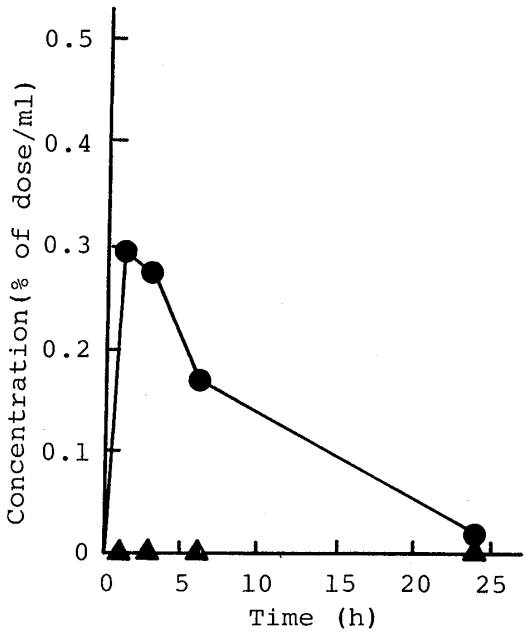

Fig. 2. Blood Concentration of ^{14}C-Labeled Lipid after Intramuscular Administration of Liposome and O/W Emulsion

●, [^{14}C]dipalmitoyl phosphatidylcholine in liposomes; ▲, [^{14}C]tripalmitin in O/W emulsion.

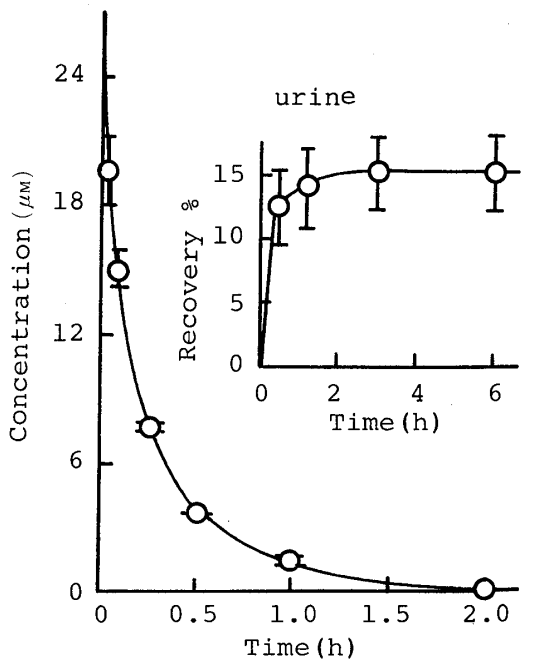

Fig. 3. Blood Concentration and Urinary Recovery of MMC after Intravenous Administration of MMC in DMSO Solution

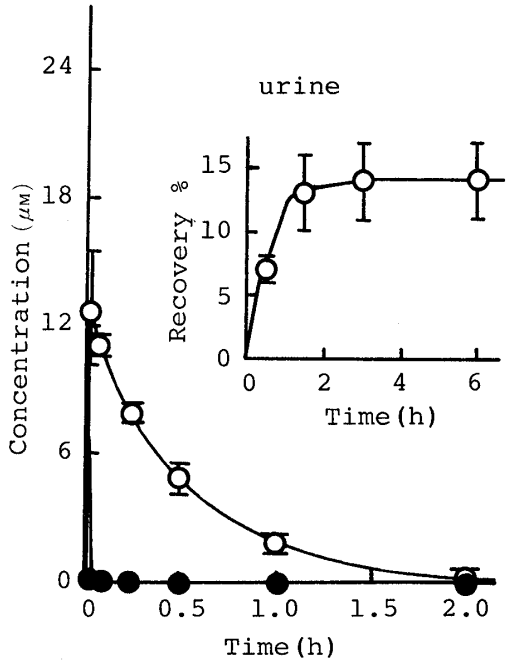

Fig. 4. Blood Concentration and Urinary Recovery of MMC and Nonyloxycarbonyl MMC after Intravenous Administration of Nonyloxycarbonyl MMC in DMSO Solution

○, MMC; ●, nonyloxycarbonyl MMC.

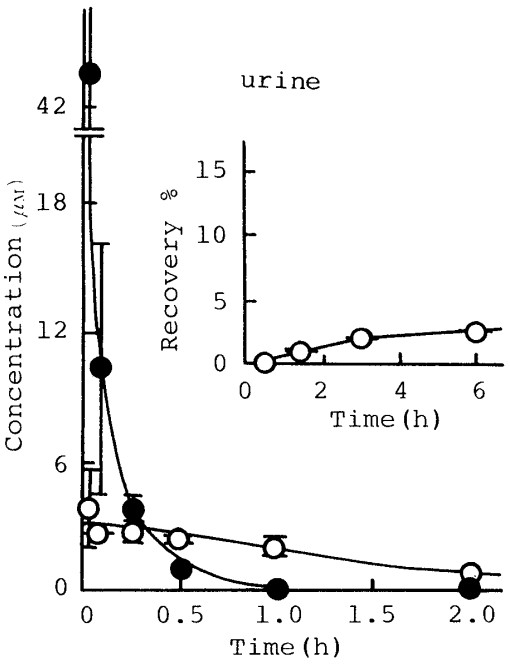

Fig. 5. Blood Concentration and Urinary Recovery of MMC and Nonyloxycarbonyl MMC after Intravenous Administration of Nonyloxycarbonyl MMC in O/W Emulsion

○, MMC; ●, nonyloxycarbonyl MMC.

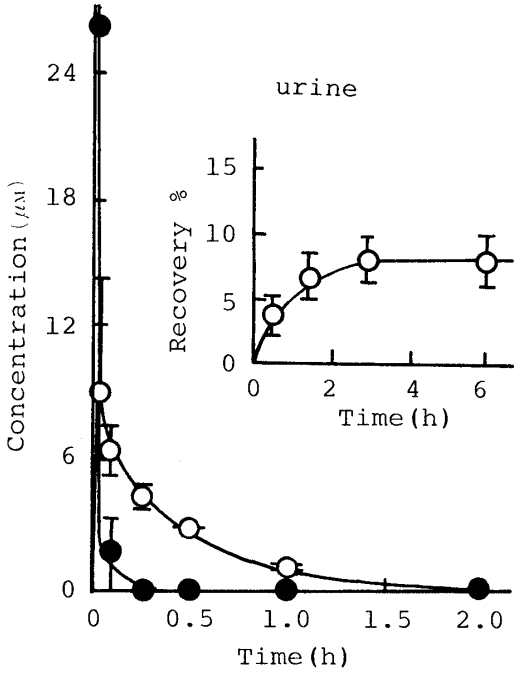

Fig. 6. Blood Concentration and Urinary Recovery of MMC and Nonyloxycarbonyl MMC after Intravenous Administration of Nonyloxycarbonyl MMC in Liposomes

○, MMC; ●, nonyloxycarbonyl MMC.

dispersion systems in the present investigation. The parent compound, MMC, having amphiphilic character, showed poor incorporation whereas nonyloxycarbonyl MMC (having fairly high lipophilicity) was stably incorporated in liposomes and O/W emulsion as described in the experimental section.

Blood Level of Drugs and Lipids after Intramuscular Administration

Figure 1 shows the blood levels of MMC and nonyloxycarbonyl MMC after administration of MMC and prodrug into the thigh muscle with liposomes and O/W emulsion. Each concentration is expressed as percent of dose/ml for comparison with lipid carrier. One percent of dose/ml is equal to 0.006 mM. MMC rapidly appeared in the blood at the early stage after injection and its concentration decreased rapidly thereafter, regardless of dosage form. On the other hand, when the prodrug was administered in the form of liposomes or O/W emulsion, neither prodrug nor parent drug could be detected in the circulation.

Figure 2 showed the blood level of labelled lipid incorporated into formulations in the same experiment. No lipid appeared in the case of emulsion, but a significant amount appeared in the case of lipids incorporated into liposomes.

Blood Level and Urinary Excretion of Drugs after Intravenous Administration

Blood levels and urinary accumulations of MMC and nonyloxycarbonyl MMC after intravenous injection in 40% DMSO–saline solution are shown in Figs. 3 and 4, respectively. After injection, MMC concentration in the blood decreased rapidly and its elimination curve showed a biexponential pattern having half-lives of 4 and 20 min in the alpha phase and beta phase, respectively. Sixteen percent of the dose was recovered in the urine at 6 h and most of that was excreted to the urine within 1.5 h. Nonyloxycarbonyl MMC disappeared rapidly immediately after injection of its DMSO solution, and MMC regenerated from the prodrug appeared in the blood at a high level. The concentration of MMC regenerated from the

prodrug decreased rapidly with a half-life equal to that of MMC administered alone in solution, and 13% of the dose was recovered in the urine only as the parent drug.

Figures 5 and 6 show blood concentration time courses and urine accumulation patterns of the prodrug and its parent drug regenerated after intravenous administration of nonyloxycarbonyl MMC with liposomes and O/W emulsion. As can be seen in Fig. 5, nonyloxycarbonyl MMC administered in O/W emulsion remained in the circulation slightly longer than that administered in DMSO solution, and MMC regenerated from the prodrug showed sustained appearance at a low level for 2 h. Slight excretion of MMC into the urine was seen, but no prodrug was detected. In the case of liposomes (Fig. 6), similar phenomena, *i.e.*, stabilization of the prodrug and sustained release of the parent drug, were observed.

Discussion

In cancer chemotherapy, it is necessary to control the pharmacokinetic behavior of the cytotoxic drug for effective treatment.[11] In particular, a sufficient supply of anticancer agents to the lymphatic system seems to offer a promising means of preventing lymph node metastasis, which has a poor prognosis. To accomplish this, we have already reported the development of several delivery systems based on a pharmaceutical or chemical approach.[12] In the previous investigation, potentially lymphotropic delivery systems of MMC were developed by combining physical and chemical approaches to overcome the low entrapping capacity of liposomes and O/W emulsion for amphiphilic MMC.[5] In the present study, the usefulness of these combined lipidic carrier systems was further demonstrated by investigation of the drug blood level after intramuscular and intravenous administration of these formulations.

MMC has been demonstrated to have antitumor activity against a number of human neoplasms including chronic myelogenous leukemia and solid tumors of various organs.[13] In clinical practice, however, toxicity problems such as delayed cumulative myelosuppression have impeded its utilization.[14] Therefore, it is important to investigate the blood level of MMC in relation to the onset of systemic side effects.

As shown in Fig. 1, no active drug appeared in the circulation after intramuscular injection of the prodrug in lipidic forms, while MMC showed high blood level at an early stage regardless of dosage form. These results suggest that lipidic formulations combined with a lipophilic prodrug might be useful in topical application to decrease the systemic side effects of MMC while maintaining a high activity of the drug at the injection site and lymph nodes, as shown in the previous papers.[5] The difference of behavior between the carrier and the prodrug in blood (Figs. 1 and 2) is considered to be due to rapid release of the prodrug from the liposomes and its subsequent conversion into MMC (Fig. 6). MMC showed rapid elimination and poor urinary recovery when administered in the form of DMSO solution as shown in Fig. 3. This result is in good agreement with those reported previously.[15] MMC was reported to show a short half-life in the plasma owing to extensive metabolism by reductive enzymes in the liver.

In the case of the prodrug, the conversion to MMC in the plasma and liver homogenate has already been reported kinetically *in vitro*.[8] In the present *in vivo* experiment, it was confirmed that the prodrug is converted rapidly to MMC in the body immediately after intravenous administration (Fig. 4).

In contrast to these results, the administration of the prodrug in a lipid dispersion system resulted in the sustained appearance of active drug, MMC, suggesting stabilization of the prodrug in the blood (Figs. 5 and 6), as reported previously.[7] However in practical use there remain some problems such as rapid removal of the drug or disruption of carriers by attack of plasma protein, high density lipoprotein, and other biological components.[16] Therefore, for

the optimal sustained release of MMC, it might be necessary to select a suitable prodrug and stable lipid components.

Thus, the integrated approach, *i.e.*, combined use of physical and chemical modifications, seems to offer a promising means of optimization of drug delivery. We have assessed the design factors for such integrated delivery systems and have successfully developed another lymphotropic delivery sytem for the antitumor antimetabolite 5-fluorouracil, in the same manner as described above for MMC. The results will be described in detail in subsequent papers.

Acknowledgement This work was supported in part by a Grant-in-Aid for Cancer Research (56-10) from the Ministry of Health and Welfare, and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References

1) G. Gregoriadis, *Nature* (London), **283**, 814 (1980); H. M. Patel and B. E. Ryman, "Liposomes; From Physical Structure to Therapeutic Applications," ed. by C. G. Knight, Elsevier/North-Holland Biomedical Press, Amsterdam, 1981, pp. 409—436.
2) M. J. Koslosky, F. Rosen, R. J. Milholland, and D. Papahadjopoulos, *Cancer Res.*, **38**, 2848 (1978); R. L. Juliano and D. Stamp, *Biochem. Pharmacol.*, **27**, 21 (1978); A. Rahman, N. More, and P. S. Schein, *Cancer Res.*, **42**, 1817 (1982); A. W. Segal, G. Gregoriadis, and C. D. V. Black, *Clin. Sci. Mol. Med.*, **49**, 99 (1975).
3) C. G. Knight, "Liposomes; From Physical Structure to Therapeutic Applications," ed. by C. G. Knight, Elsevier/North-Holland Biomedical Press, Amsterdam, 1981, pp. 381—389.
4) F. C. Szoka and D. Papahadjopoulos, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 4194 (1978); J. H. Fendler, "Liposomes in Biological Systems," ed. by G. Gregoriadis and A. C. Allison, John Wiley and Sons, New York, 1980, pp. 87—100.
5) H. Sasaki, Y. Takakura, M. Hashida, T. Kimura, and H. Sezaki, *J. Pharmacobio-Dyn.*, **7**, 120 (1984); H. Sasaki, T. Kakutani, M. Hashida, and H. Sezaki, *J. Pharm. Pharmacol.*, in press.
6) L. H. Baker, F. M. Caoli, R. M. Izbicki, M. I. Opipari, and V. M. Vaitkevicins, *Proc. Am. Assoc. Cancer Res.*, **15**, 182 (1974); S. T. Crooke and W. T. Bradner, *Cancer Treat. Rev.*, **31**, 121 (1976).
7) S. B. Kaye and V. J. Richardson, *Cancer Chemother. Pharmacol.*, **3**, 81 (1977); R. L. Juliano and D. Stamp, *Biochem. Pharmacol.*, **27**, 21 (1978); H. K. Kimelberg and M. L. Atchison, *Ann. N. Y. Acad. Sci.*, **308**, 395 (1978).
8) H. Sasaki, E. Mukai, M. Hashida, T. Kimura, and H. Sezaki, *Int. J. Pharmaceut.*, **15**, 49 (1983); *idem, ibid.*, **15**, 61 (1983); H. Sasaki, M. Fukumoto, M. Hashida, T. Kimura, and H. Sezaki, *Chem. Pharm. Bull.*, **31**, 4083 (1983).
9) T. Tanaka, K. Taneda, H. Kobayashi, K. Okumura, S. Murahishi, and H. Sezaki, *Chem. Pharm. Bull.*, **23**, 3069 (1975).
10) A. D. Bangham and R. W. Horne, *J. Mol. Biol.*, **8**, 660 (1964).
11) G. Gregoriadis, *Nature* (London), **265**, 407 (1977); R. L. Juliano (ed.), "Drug Delivery Systems," Oxford University Press, New York, 1980.
12) M. Hashida, M. Egawa, S. Muranishi, and H. Sezaki, *J. Pharmacokinet. Biopharm.*, **5**, 225 (1977); H. Sezaki, M. Hashida, and S. Muranishi, "Optimization of Drug Delivery," ed. by H. Bundgaard, A. B. Hansen, and H. Kofod, Munksgaard, Copenhagen, 1982, pp. 241—255; T. Yoshioka, M. Hashida, S. Muranishi, and H. Sezaki, *Int. J. Pharmaceut.*, **8**, 131 (1981); A. Kato, Y. Takakura, M. Hashida, T. Kimura, and H. Sezaki, *Chem. Pharm. Bull.*, **30**, 2951 (1982); Y. Takakura, S. Matsumoto, M. Hashida, and H. Sezaki, *Cancer Res.*, **44**, 2505 (1984).
13) C. Everett, *Ann. Surg.*, **160**, 95 (1964); R. L. Comis and S. K. Carter, *Cancer*, **34**, 1576 (1974).
14) S. T. Crooke, "Mitomycin C; Current Status and New Developments," ed. by S. T. Carter and S. T. Crooke, Academic Press, New York, 1979, pp. 1—4.
15) H. Fujita, *Jpn. J. Clin. Oncol.*, **12**, 151 (1971); S. D. Reich, "Mitomycin C; Current Status and New Developments," ed. by S. K. Carter and S. T. Crooke, Academic Press, New York, 1979, pp. 243—250.
16) G. Scherphof, J. Damen, and D. Hoekstra, "Liposomes; From Physical Structure to Therapeutic Applications," ed. by C. G. Knight, Elsevier/North-Holland Biomedical Press, Amsterdam, 1981, pp. 299—321.