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Absorption characteristics of the lipophilic prodrug of mitomycin C from injected liposomes or an emulsion

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Mitomycin C (MMC) is amphiphilic and so cannot be incorporated into lipoidal delivery systems. To develop a lipoidal delivery system, its prodrug, nonyloxycarbonyl MMC, was formulated in liposomes and in o/w emulsions and the usefulness of these formulations was evaluated. After injection into the rat thigh muscle, MMC was rapidly absorbed regardless of the dosage form. However, the prodrug was retained at the injection site for considerably longer when formulated in a lipid dispersion system. The accumulation of MMC at regional lymph nodes was also investigated and whereas free MMC arrived at and disappeared from the lymph nodes almost immediately after injection, the prodrug arrived at an early stage and its concentration decreased only gradually, remaining fairly high 2 h after injection. Liposomal lipids appeared to accumulate at the lymph nodes to a greater degree than o/w emulsions. It is suggested that the combination of lipidic carrier devices with lipophilic prodrugs may be a useful adjunct to cancer chemotherapy.

Many attempts have been made to deliver cytotoxic drugs to the tumour site by means of drug delivery systems (Gregoriadis 1977; Juliano 1980). We have been engaged in studies on cancer drug delivery systems which either utilize physical devices (Hashida et al 1977, 1979; Yoshioka et al 1981) or chemically transform the drug molecules to prodrugs (Kojima et al 1980; Hashida et al 1981; Kato et al 1982; Sasaki et al 1983a, b, c). Of the physical devices, emulsified dosage forms have been clinically investigated and positive results have been achieved (Tanigawa et al 1980; Sezaki et al 1982).

However, further optimization of drug delivery and the resultant improvement in drug efficacy may be achieved through rational systematic application of available pharmaceutical techniques such as dosage form design, chemical modification and alteration of dose regimen. On the basis of this, we have designed promising delivery systems for anticancer agents by combining physical and chemical approaches. To overcome the difficulties posed by liposomes and o/w emulsions, which cannot entrap amphiphilic compounds like most anticancer agents, chemical approaches have been introduced into the design of lipidic delivery systems.

In a previous study, we synthesized several derivatives of the cytotoxic antibiotic, mitomycin C (MMC), and confirmed that their characteristics and antitumour activities were due to a MMC prodrug (Sasaki et al 1983a, b, c). Furthermore, our recent study showed that of the prodrugs investigated, nonyloxycarbonyl MMC seemed to be the best one for application to lipid dispersion systems, and showed the highest feasibility for encapsulation in them while retaining its antitumour activity in-vivo (Sasaki et al 1984).

In the present study, the fate of liposomes and o/w emulsions incorporating MMC and its lipophilic prodrug, nonyloxycarbonyl MMC, was investigated using the rat thigh muscle as a model injection site for evaluating the usefulness of these combined systems as lymphotropic delivery and local sustained release systems in cancer chemotherapy.

MATERIALS AND METHODS

Materials

MMC was supplied from Kyowa Hakko Kogyo Co. The lipophilic derivative, nonyloxycarbonyl MMC, was synthesized by the method of Sasaki et al (1983c). Egg phosphatidylcholine was prepared from egg yolks by a method described by Tanaka et al (1975). Nonionic surfactant, a polyoxyethylene derivative of hydrogenated castor oil (HCO-60), was obtained from Nikko Chemicals Co. Radio-labelled lipids, [^{14}C]dipalmitoyl phosphatidylcholine and [^{14}C]tripalmitin, were purchased from the Japan Radioisotope Association. All other chemicals were of reagent grade and obtained commercially.

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Preparation of liposomes and o/w emulsion

Liposomes were prepared by the method of Bangham & Horne (1964) using egg phosphatidylcholine. Phosphatidylcholine and drug, dissolved in chloroform at the molar ratio of 4:1, were evaporated under vacuum to a thin lipid film. The dry lipid film was then suspended in a saline solution by Vortex shaking, and the resulting suspension sonicated at 0 °C for 3 min under nitrogen.

An o/w emulsion was prepared with 4 volumes of sesame oil containing the drug and 6 volumes of an aqueous phase containing a surfactant (7·5 v/v% HCO-60). Emulsification was by sonication.

Both formulations had a final drug concentration of 5·98 mM.

Animal experiments

Male Wistar albino rats (200–220 g) were temporarily anaesthetized by ether and injected with 100 µl of the formulations into the right thigh muscle. At various times after injection, the rats were killed and the muscle and the regional lymph node excised, homogenized in a Teflon homogenizer and diluted to constant volumes. Ethylacetate was added to aliquots of the homogenate samples, to aid extraction of the nonyloxycarbonyl MMC. The organic layer was evaporated and the residue dissolved in a little DMSO for assay of the prodrug using high performance liquid chromatography (HPLC). Another aliquot was washed with chloroform and the aqueous phase was reserved for bioassay of MMC.

Bioassay

Bioassay of MMC was carried out by a paper disc method using *Escherichia coli B* as a test organism. The antimicrobial activity was determined by measuring the diameter of the growth inhibitory zone after 24 h incubation at 37 °C following 24 h for diffusion of the drug in agar.

HPLC assay

Nonyloxycarbonyl MMC was determined using an HPLC system (TRIROTAR, Jasco) equipped with a variable wavelength uv-detector (UVIDEC 100-II, Jasco). The stationary phase used was a Cosmosil $5C_{18}$ packed column (4·6 × 150 mm, Nakarai Chemicals) and 80% methanol in water was used as the mobile phase with a flow rate of 0·8 ml min^{-1}. Chromatograms of the standard solutions were obtained and calibration lines were constructed on the basis of peak-area measurements.

Radioisotope experiment

Lipids incorporating [^{14}C]dipalmitoyl phosphatidylcholine and [^{14}C]tripalmitin were used. The procedure for preparation of the dosage forms and the animal experiments were the same as above. Lipids were detected by radioactivity assay.

Radioactivity assay

The muscle and lymph node homogenates were digested at 45 °C in Soluene 350 (Packard Instrument Co., Sowners Grove, Ill.) and then decolourized with 100 µl of benzoyl peroxide. Samples were analysed for ^{14}C content 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

The structure and physicochemical properties of nonyloxycarbonyl MMC and MMC are listed in Table 1. The prodrug showed a partition coefficient which was higher than that of MMC by a factor of ten thousand. It also showed increased lipid solubility and decreased aqueous solubility. MMC was scarcely incorporated into lipoidal formulations such as liposomes and o/w emulsions, while the prodrug,

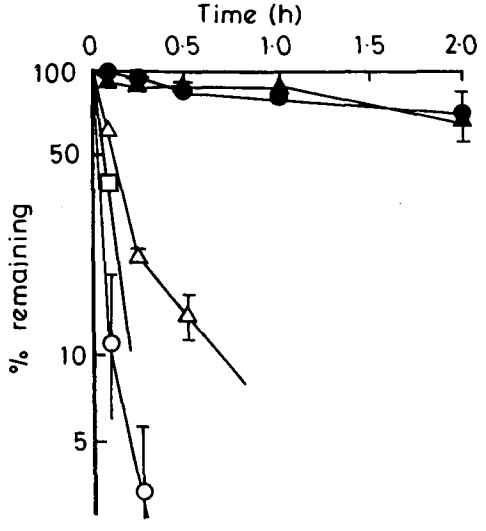
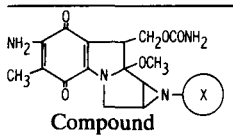


FIG. 1. Disappearance of MMC or nonyloxycarbonyl MMC from the rat thigh muscle after intramuscular injection in liposomes and o/w emulsion. □, MMC in saline; ○, MMC in liposomes; △, MMC in o/w emulsion; ●, nonyloxycarbonyl MMC in liposomes; ▲, nonyloxycarbonyl MMC in o/w emulsion.

Table 1. Structures and physicochemical properties of the lipophilic mitomycin-C prodrug.



Compound	X	PC_{oct}	Solubility (37 °C) Water (mg ml−1)	Solubility (37 °C) Sesame oil (mg ml^{-1})	Entrap percent Liposome	Entrap percent o/w Emulsion
Mitomycin C (MMC)	-H	0·41	0·912	0·006	0·1	1·0
Nonyloxycarbonyl MMC	$-COO-C_9H_{19}$	3637	0·002	7·646	99·9	99·9

having higher lipophilicity, exhibited complete incorporation.

Fig. 1 shows the disappearance of the lipophilic prodrug, nonyloxycarbonyl MMC, from the thigh muscle after intramuscular injection of the drugs as liposomes or o/w emulsion. The absorption profiles of free MMC, administered as an aqueous solution, and lipidic dosage forms are illustrated for comparison. MMC was rapidly absorbed from the injection site regardless of the dosage form and little remained there after 30 min. However, nonyloxycarbonyl MMC was retained at the injection site for a much longer period when administered as liposomes or as an o/w emulsion. More than 70% of the dose remained as the prodrug, in the muscle even at 120 min after injection with negligible MMC regeneration. On the other hand, more retarded absorption was observed for lipids in carrier systems as shown in Fig. 2 since high levels of radioactivity remained at the injection site 24 h after injection.

The transfer of drugs to the regional lymph node after intramuscular injection was examined and results are shown in Fig 3. Immediately after injection, free MMC arrived at the regional lymph

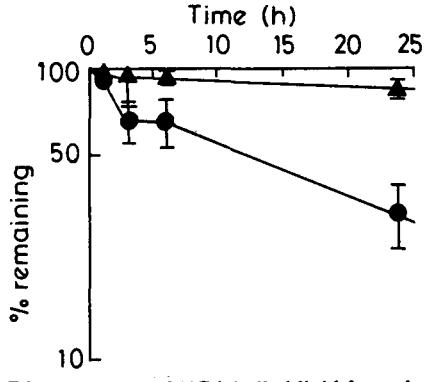


FIG. 2. Disappearance of ^{14}C-labelled lipid from the thigh muscle after intramuscular administration of liposome and o/w emulsion. ●, [^{14}C]dipalmitoyl phosphatidylcholine in liposomes; ▲, [^{14}C]tripalmitin in o/w emulsion.

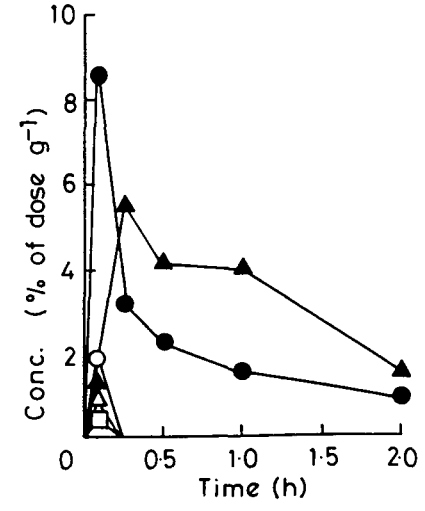


FIG. 3. Concentration of MMC or nonyloxycarbonyl MMC in the iliac lymph node after intramuscular administration in liposomes and o/w emulsion. □, MMC in saline; ○, MMC in liposome; △, MMC in o/w emulsion; ●, nonyloxycarbonyl MMC in liposome; ▲, nonyloxycarbonyl MMC in o/w emulsion.

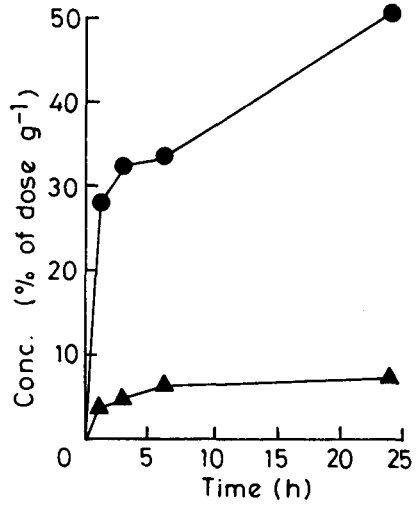


FIG. 4. Concentration of ^{14}C-labelled lipid in the iliac lymph node after intramuscular administration of liposome and o/w emulsion. ●, [^{14}C]dipalmitoyl phosphatidylcholine in liposomes; ▲, [^{14}C]tripalmitin in o/w emulsion.

nodes but then disappeared rapidly, and no active MMC was detected 15 min after injection regardless of the dosage form. Nonyloxycarbonyl MMC appeared in the lymph nodes, without detectable regenerated MMC, soon after injection and the concentration decreased gradually, but the prodrug was still present in the lymph node 2 h after injection. On the other hand, as shown in Fig. 4, an accumulation of liposomal lipid was observed in the lymph nodes, while the o/w emulsion labelled with [^{14}C]tripalmitin was also accumulated but to a lesser extent.

DISCUSSION

Recently, the use of lipid dispersion systems such as liposomes and o/w emulsions as carriers of antitumour drugs has been extensively developed (Gregoriadis 1980; Patel & Ryman 1981). The usefulness of encapsulating chemotherapeutic agents in lipidic carriers has been demonstrated in systemic and local administration (Koslosky et al 1978; Juliano & Stamp 1978; Rahman et al 1982; Segal et al 1975).

However, there is a drawback to the application of certain drugs in these lipid dispersion systems. Amphiphilic compounds, like MMC, are poorly entrapped in these carrier systems while highly lipophilic or hydrophilic compounds can be efficiently incorporated in them (Knight 1982). Although numerous efforts to improve the entrapment with liposomes have been attempted (Szoka & Papahadjopoulos 1978; Fendler 1980), by manipulation of lipid composition, buffer contents or preparation procedures, no conclusive improvement has been accomplished.

There is another approach. By chemical modification, many drugs can be given properties which control their entrapment and retention by a lipid carrier. In fact, as shown in Table 1, the amphiphilic MMC was poorly incorporated in lipoidal dosage forms, while nonyloxycarbonyl MMC, being extremely lipophilic, due to the introduction of lipophilic functions to the MMC molecule, was highly incorporated in them.

In the present study, the usefulness of combining a lipidic formulation with a lipophilic prodrug as a local administration delivery system was evaluated using the rat thigh muscle as the model injection site.

By local administration of the encapsulated drug directly into a single cancerous target organ, the antitumour drug could be constrained and so exert its pharmacological effects within this organ (McCullough & Juliano 1979). Indeed, the lipidic carrier system remained for long periods at the injection site as shown in Fig. 2, and administration of the lipophilic prodrug with a lipid carrier causes the prodrug to remain effectively at the injection site. However, in spite of the retention of the lipid carrier, MMC disappeared rapidly, regardless of dosage form.

The lymphotropicities of drugs and lipids are shown in Figs 3 and 4. In cancer chemotherapy, a sufficient supply of anticancer agents to the lymphatic system seems to offer a promising means of preventing lymph node metastasis. In our prodrug preparations, accumulation of labelled lipid as carrier and high availability of prodrug in the regional lymph node was observed after intramuscular injection compared with MMC.

These results indicate that application of the lipophilic prodrug, but not MMC itself, as liposomes or o/w emulsions could cause MMC to be retained at the injection site and then delivered to target sites, such as lymph nodes, depending on the properties of the carrier after local administration.

On the other hand, the observed difference in behaviour between drugs and lipid carriers might indicate the existence of a drug releasing process from liposomes or o/w emulsions depending on their lipophilicity at the injection site and at the lymph node. The free nonyloxycarbonyl MMC released in the body seemed to be rapidly converted to MMC by enzymes and the regenerated MMC should exhibit its cytotoxic action as described previously (Sasaki et al 1983a, b, c). Knowledge of this behaviour of the antitumour agent will be useful in the formulation of sustained release and lymphotrophic delivery systems for local injection after surgical treatment to prevent lymphatic metastases.

Thus the combined use of physical and chemical modifications seems to offer a promising future in improving drug delivery systems.

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