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General Review

Potential of Liposomes as Drug-Carriers in Cancer Chemotherapy: A Review

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Summary. *Liposomes are bilayered phospholipid vesicles that have been proposed as vehicles for the selective delivery of cytotoxic drugs into malignant cells. In vitro and in vivo experiments have indicated that the activity of a range of drugs or their active metabolites may be enhanced by encapsulation in liposomes, particularly when used against drug-resistant tumours. Moreover, liposomal entrapment certainly has a marked effect on the tissue distribution and rates of clearance of cytotoxic drugs, and also appears to reduce their toxicity in most cases.*

However, in both animal and patient studies, the major sites of uptake following IV administration consistently appear as the liver and spleen. Preferential tumour uptake has therefore not yet been achieved, although a degree of localization of liposomal labels can be demonstrated in the vicinity of experimental animal tumours in certain circumstances. Liposomes may have a future role in cancer chemotherapy, but much laboratory work remains to be done before clinical application can be considered.

Introduction

Modern cancer chemotherapy currently offers the prospect of cure for patients with certain metastatic malignancies, but its application is still limited in the majority of solid tumours. One of the major problems is the toxicity of anticancer drugs to normal tissues, and in some cases this may be more profound and longer-lasting than any effect on the tumour. Much effort is therefore directed towards increasing drug selectivity, and the development of new drugs is particularly important in this respect. However, there is still considerable scope for improving the results obtained with the drugs that are available at present. Several methods have been suggested for the selective delivery of cytotoxic drugs to malignant cells [9, 47] though none has yet received widespread clinical acceptance. One approach has been the use of liposomes as versatile carriers of cytotoxic drugs.

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Liposomes and Drug Entrapment

Liposomes are closed bilayered vesicles, which form readily when phospholipids are dissolved in organic solvents (chloroform/methanol) and rotary-evaporated under vacuum to a thin layer, then allowed to swell in aqueous solutions [3]. They have a concentric multilamellar structure (see Fig. 1) and are approximately 1 μm in diameter; their physical and chemical properties (including membrane fluidity and charge) can be varied by use of the appropriate lipid components in the initial preparation.

The composition used consists of a phospholipid, usually phosphatidyl choline (lecithin), to which may be added cholesterol (which decreases membrane fluidity and permeability) and charged amphiphiles such as stearylamine or phosphatidic acid if positively or negatively charged liposomes are required [50]. Furthermore, multilamellar liposomes may be reduced in size by sonication, to form unilamellar vesicles with a diameter of about 25 nm.

Entrapment of cytotoxic drugs is readily performed, either by including the drug dissolved in organic solvent in the initial solution prior to evaporation, or by dissolving the drug in the aqueous solution used at the stage of lipid dispersion. Several agents have now been encapsulated by these means (see Table 1). The amount of drug incorporation and the degree of retention within the vesicles are dependent on the physicochemical properties of the drug concerned and the lipid composition of the liposomes used. A particularly important factor is the oc-

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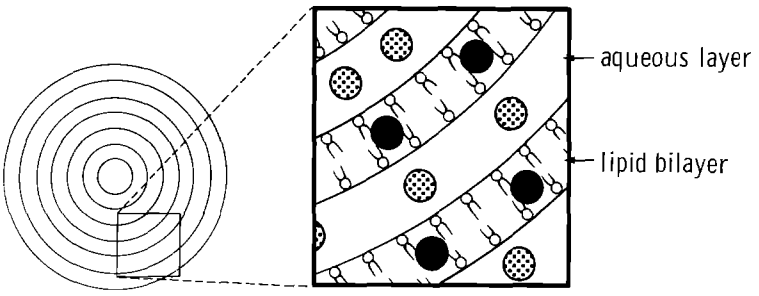

Fig. 1. Schematic representation of a multilamellar liposome (approximate diameter 1 μm). ●, lipid-soluble molecule; ⊖, water-soluble molecule

Table 1. Cytotoxic drugs entrapped in liposomes

Drug	References	Drug	References
Drugs entrapped in the aqueous phase of liposome structure			
Actinomycin-D	12, 13, 36	6-Mercaptopurine	49
Methotrexate	6, 24	8-Azaguanine	49
Cytosine arabinoside	23, 25, 27	Melphalan	19
5-Fluorouracil	12	Mechlorethamine	41
L-Asparaginase	29	Bischloroethylnitrosourea	41
Drugs entrapped in the lipid phase of liposome structure			
Actinomycin-D	12, 23, 36	*cis*-Platinum	7
Daunomycin	23	Bleomycin	16
Vinblastine	23		

tanol-water partition coefficient of the drug to be entrapped. Examples of nonpolar drugs with relatively high entrapment efficiencies are vinblastine, adriamycin, and actinomycin-D [23], while drugs such as cytosine arabinoside and 5-fluorouracil show lower entrapment and higher rates of leakage [13, 23].

Recently an alternative method of preparation of unilamellar liposomes based on fast dialysis has been suggested [28]. As well as providing a high yield of homogeneous vesicles, the method might permit entrapment of some cytotoxic agents, particularly those of low molecular weight, with greater efficiency.

Behaviour of Liposomes in vitro and in vivo

Liposomes were originally developed during the 1960s as a model for the study of cell membranes [2] and it has been relatively recently that they have been proposed as a means of delivery of cytotoxic drugs [14, 31]. The exact process of interaction of liposomes with cells is not fully understood, but it is believed that they have the capacity to deliver their contents to the cell interior, either by endocytosis and subsequent interaction with intracellular lysosomes, or by fusion with the cell membrane [30, 33]. It has been shown that certain malignant cells possess a greater endocytic capacity for macromolecules than normal cells [10, 48] and it was therefore suggested that liposomes might provide a means of increasing the delivery of cytotoxic drugs to their target, i.e., cancer cells.

Encouraging results from in vitro experiments have raised hopes of producing such a selective drug carrier, since it has been demonstrated that drugs entrapped in liposomes gain ready access to cultured tumour cells [34]. Other workers have shown that this uptake may be enhanced by the incorporation of suitable antisera in the liposomal bilayer, using as the model HeLa cells and AKR-A cells in culture [16].

Investigations on the in vivo distribution of liposomes have largely been performed indirectly by the incorporation of radioactive labels such as bleomycin ^{111}Indium [16] or technetium ^{99}m [37]. The evidence from these studies supports the suggestion that liposomes do localize to some extent in the vicinity of animal tumours; moreover the size and charge of the lipo-

somes and the time interval after injection appear to be important factors in determining the degree of uptake [20, 38]. Indeed, no evidence of tumour localization of technetium ^{99}m-labelled liposomes was found in a separate study in tumour-bearing mice, where these experimental conditions were not individually examined [1].

However, at present selective drug delivery to tumours in vivo remains elusive, since uptake of liposomes following IV injection is carried out predominantly by the cells of the fixed reticuloendothelial system in the liver and spleen. Attempts to increase the targeting ability of liposomes in vivo by incorporation of tumour-associated antibody have been reported [20]. However, they have met with little success, for although it was claimed that liposomes bearing such antibody did localize to a slightly greater extent in the animal tumours examined, they were still preferentially taken up by the liver and spleen. So far mechanisms aimed at blocking the uptake by the reticuloendothelial system have made little impact [15, 46]. In cases where a degree of localization of liposomes in experimental solid tumours has apparently occurred, no direct morphological evidence of uptake is yet available. The exact site of localization, if any, within the tumour is therefore not known, and in particular the role of host cells such as macrophages is not understood. A better appreciation of the mechanisms involved is clearly necessary for proper analysis of therapeutic studies.

The investigations on the in vivo fate of liposomes have been extended to follow their tissue distribution following IV injection to patients with tumours. In early reports [18] it was suggested that liposomes were preferentially taken up by malignant deposits, when albumin ^{131}I was used as the tracer. However, it is known that many malignant cells have an enhanced capacity for the endocytosis of free albumin [4] and when liposome localization studies in patients were repeated with bleomycin ^{111}In no preferential tumour uptake was demonstrated [44]. Similar negative conclusions were reached in a study of patients with a range of tumours, using technetium ^{99}m-labelled liposomes followed visually with a gamma camera [42]. No obvious toxicity has been apparent in these preliminary studies on patients, though further work is clearly required with liposomes of a different composition. The distribution of technetium ^{99}m-labelled liposomes following interstitial injection has also been examined, with particular reference to the role of regional lymph nodes. Data from animal experiments with liposomes of various charges have shown that the pattern of uptake of the vesicles in regional lymph nodes draining a primary tumour differs from that seen in normal lymph nodes [39]. Similar phenomena appear to apply in patients with tumours (M. P. Osborne, personal communication), and this approach may have considerable diagnostic potential in lymphoscintigraphy.

Therapeutic Possibilities

It is evident that no substantial degree of selective localization of liposomes in tumours has yet been achieved, though further modifications, particularly with the incorporation of more specific tumour-associated antibody, may be more successful. Nevertheless, liposomes do possess properties that suggest they could still have a role in enhancing the efficacy of certain antitumour drugs. One such capacity is the marked effect that liposomes have on the pharmacokinetics and tissue distribution of injected drugs [23]. For example, the clearance of actinomycin-D from the circulation, normally a rapid process occurring within minutes, is considerably prolonged in experimental animals when the drug is administered entrapped in liposomes, with 45%—50% of the injected dose present 3 h after injection [12, 23]. The precise form in which the entrapped drug circulates after injection is not known, nor is there adequate information on its cytotoxic activity while in the liposome-entrapped form. The in vitro interactions of liposomes with serum components have been studied in some detail, and it is evident that considerable disruption occurs. High-density lipoproteins are particularly involved in the process of complex formation and disintegration of the vesicles [43, 45] while the α and β globulin fractions have also been implicated in cellular uptake of liposomes as well as leakage of entrapped drugs [51].

Nevertheless, if the entrapped drug does remain in the circulation in a biologically active form, the prolongation of the plasma half-life might carry therapeutic advantages. For S phase-specific drugs such as cytosine arabinoside there is already clinical evidence that prolonged exposure produces optimum therapeutic effects [8]. For other drugs, the evidence for a therapeutic advantage is less clear; indeed, prolonged administration in some cases may in fact result in increased toxicity.

The altered rate of clearance of liposome-entrapped drugs has been shown to vary according to the size and the surface charge of the vesicles used [22]. The same factors also influence the altered tissue distribution of the entrapped drugs [21] and this may also carry therapeutic benefits. With respect to normal tissues, liposome encapsulation of most drugs so far studied results in reduced accumulation in intestinal wall, kidney, and cardiac muscle [12, 23]. The dose-limiting toxicity of most cytotoxic drugs is manifest mainly in these organs and in bone marrow, and it seems conceivable that selective reduction of uptake in specific areas by means of liposome-encapsulated drugs may reduce this toxicity. Animal experiments so far reported have confirmed that the toxicity of a drug such as actinomycin-D is indeed reduced by entrapment, and this appears to result from a sparing effect on both proliferating intestinal cells and the stem cells of the bone marrow [36].

The corollary of this reduction in toxicity is that the antitumour activity of the entrapped form of the drug should not be similarly reduced, as otherwise there may be no overall benefit, as estimated by the therapeutic index. Several comparative studies on the cytotoxic effects of free and entrapped drugs have been reported, and some workers claim that the efficacy of a drug such as actinomycin-D is actually enhanced when it is encapsulated in liposomes [17, 35]. Similar results have also been obtained in studies with other drugs, including bischloroethylnitrosourea and mechlorothamine [41] and cytosine arabinoside [25, 27]. However, the experimental tumours studied were limited to the Ehrlich ascites tumour [35, 41], L1210 leukaemia [25, 27, 41], and the AKR-A lymphoma [17], grown as an ascites tumour. It was suggested that the encouraging results obtained might indicate increased drug uptake by malignant cells, but there is little evidence that this occurs, and it is conceivable that the therapeutic effects observed resulted simply from a prolongation of the plasma half-life or a reduction in toxicity for normal tissues of the drugs used.

A specific area of experimental chemotherapy where drug entrapment in liposomes has proved more promising is the treatment of drug-resistant tumours. It is well established that malignant cells may develop resistance to several cytotoxic drugs, e.g., actinomycin-D, as a result of reduced transport across the cell membrane [40] and it was hoped that liposomes might prove capable of bypassing this permeability barrier. In vitro experiments with entrapped actinomycin-D in a resistant Chinese hamster cell line confirmed that this mechanism is feasible [32]. A 200-fold reduction in the dose of drug required to produce similar inhibitory effects was demonstrated when the activity of the entrapped drug was compared with its activity in the free state. At present, however, there is no evidence that actinomycin-D resistance in vivo can be similarly overcome. Nevertheless, a recent study has demonstrated a marked benefit in favour of liposome-entrapped methotrexate over free drug in the treatment of a solid rodent tumour that was relatively resistant to free methotrexate [26]. The mechanisms underlying this important finding have not yet been elucidated and it is possible that a prolongation of the plasma half-life of methotrexate was a critical factor. Additional studies of this apparent enhanced effectiveness of the drug are clearly indicated.

A further possible therapeutic role for liposomes has been suggested by experiments in which the active metabolite of the cytotoxic drug – cytosine arabinoside – has been entrapped in the vesicles and tested in vitro [27]. The activity of cytosine arabinoside depends partly on intracellular phosphorylation to the active metabolite – the triphosphate (Ara CTP) by the enzyme cytidine kinase [5]. This rate-limiting step may theoretically be by-passed by direct introduction of Ara CTP into target cells by means of liposomes, and results with L1210 cells in culture have indeed demonstrated a therapeutic advantage in favour of liposome-entrapped Ara CTP over free Ara CTP [27].

In summary, as information on the mechanisms of cellular resistance to cytotoxic drugs continues to accumulate [11] the potential of liposomes as unique vehicles for those agents assumes added significance.

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