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TARGETING OF TUMOR CELLS BY LIPOSOMES –ENCAPSULATED MISONIDAZOL

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ABSTRACT

The effect of liposomal drug encapsulation on the distribution pattern of the encapsulated drug, in tumor and normal tissues of tumor-bearing mouse, was studied . The radioactivity content in some selected organs and serum , after administration of ^{14}C - labelled misonidazole, was followed at different time intervals. Depending on liposomal the drug formulation type, the uptake of the drug into normal tissues and serum was found to be variable and could be arranged in the following descending order: drug free form > small unilamellar liposomally encapsulated drug > multilamellar liposomally encapsulated drug. On the contrary, the uptake of the drug into tumor tissues was greatest for multilamellar liposomal form, less for small unilamellar liposomes and least for drug free form. Also, the half-time ($t_{1/2}$) for drug clearance was longest for multilamellar liposome, less for small unilamellar liposome and least for drug free form. However, for both liposomal types, the half-time for drug clearance was longest for positively charged liposomes and least for negatively charged type. It could be concluded that multilamellar liposomes with positive surface charge represented an optimal or most suitable formulation for modulating drug action in cancer therapy and targeting the drug to the selected site of action .

INTRODUCTION

The goal of any drug delivery system involves the development of target oriented selective carriers for altering the pharmacokinetics and physiological disposition of the drug in question in order to obtain a higher therapeutic index. It was therefore suggested that the ideal drug carriers should be biodegradable, nontoxic and should be able to encapsulate and protect various drug molecules, thereby sequestering them from the surrounding medium. Furthermore, the carrier should interact as selectively as possible with a specific receptor localized at the target tissues, and should be able to deliver the active encapsulated therapeutic agent specifically to its site of action.

Liposomes (phosholipid vesicles) have been proposed as carriers of materials of therapeutic interest such as antibiotics, antitumor drugs, enzymes, antigens and antibodies [1-10] Liposomal encapsulation of drugs can potentially overcome many problems as drug resistance, immune reactions, metabolic breakdown of molecules, drug solubility, drug stability and inability of many active drugs to reach diseased areas or homing of drugs to target tissue [11-15]. In this regard, it was postulated that the potential use of liposomes, as a particulate drug delivery systems, was related to an expected protection of the encapsulated molecules in the blood stream, and an increased uptake into cells by mechanisms that are not normally available for these drugs [16-20].

Apparently, liposomes seems to be a promising approach, of many potential benefits and advantages for controlling and sustaining drug release within the site of action and at a rate regulated by alterations in the lipid composition and variations in the liposomal formulation methods. For instance, incorporating stearylamine and dicetyl phosphate to impart either a positive or a negative charge to liposomes conveniently altered the net surface charge of liposomes. On the other hand, inclusion of lipids that forms more rigid bilayer such as cholesterol resulting in a relative prolonged release effect [21-23].

The possibility of utilizing liposomes , as a drug carrier , in tumor therapy has received great attention and has been summarized in many articles [24-30]. An approach for enhancing the effectiveness of anticancer drugs was to alter their tissue distribution and clearance from the blood by incorporating them into particulate carriers such as liposomes [25-30]. It was suggested that simple modification or manipulation of liposomal chemical composition, size, surface charge, number of lamellae and bilayer rigidity causes alterations in organ disposition, and may help to increase localization of the encapsulated drug where they can be directed toward certain target structures. Consequently, the optimal liposomal design, as a "functionalized" carrier for therapeutic applications, can be modulated depending on the physicochemical properties of liposome-drug combination [1,23,31-33].
Misonidazole, 2-nitro-imidazole derivatives, is one of the most widely used chemical radiosensitizers [34-36]. Among the different types of chemical radiosensitizers, misonidazole is the drug of choice that showed pharmacological properties very near to the ideal properties of the theoretically proposed radiosensitizers[37,38]. Accordingly, misonidazol gained a lot of attention as a promising radiosensitizer and is currently used successfully in many clinical trials for the treatment of some types of human tumors [35,39,40].

In vitro and / or in vivo misonidazole showed radiosensitizing effect and the degree of response was dependents on the degree of oxygenation of the particular solid tumor, especially it is proved that all

tumors contain certain percentage of hypoxic cells. It sensitizes specifically hypoxic tumor cells by forming a cytotoxic intermediates that may selectively kill tumor cells [34,37,41].

In this study, attempts have been made to formulate a liposomal design that will enable the drug to reach the desired site of action at a controlled rate and duration of action. Therefore, the appropriate choice of liposomal lipid components may circumvent many difficulties to enhance the ability of the encapsulated liposomal drug to interact as selectively as possible with a receptor localized at the tumor cells and not react with any other normal cells. In addition, it seems possible that simple modifications of liposomal size, charge, and composition may help to increase localization of the encapsulated drug in specific target tissues. In the following experiment, to ensure the reproducibility and comparability of experimental conditions, liposomes were prepared by techniques allowing formation of vesicles of defined size, lipid composition and surface charges.

The aim of this study was to follow and investigate: (**A**) the distribution pattern of ^{14}C – labelled misonidazole in some selected organs of tumor - bearing mice following intraperitoneal administration., and also (**B**) the pattern of distribution of the encapsulated liposomal drug. These approaches have been tried in attempts to determine if liposomal drug encapsulation can enhance the pharmacological efficacy of the encapsulated substances by altering their tissue disposition and increasing their uptake into specific target cells.

EXPERIMENTAL

Materials:

L-α-dipalmitoyl phosphatidylcholine , dicetly phosphate, stearylamine and cholesterol were obtained from Sigma Chemical Co., St., Louis, Mo.,USA. Polycarbonate membranes and membrane holders were obtained from Nucleopore Corp. 1-(2-nitro-imidazol-1-yl) - 3-methoxy propane -2-ol (misonidazole) a Roche product (Ro - 07- 0582) and also ^{14}C - labelled misonidazole were kindly supplied through the courtesy of Dr. M. M. El- Merzabani , prof. and Chairman of Cancer Biology Dept., National Cancer Institute, Cairo University. All other chemicals were of a highest grade commercially available.

Experimental animals:

Female Swiss Albino mice weighing 18-20 gram, were obtained from the breading unit of the National Cancer Institute. Cairo University, Egypt. The animals were kept in specially designed cages and fed on standard rodent pellets.

Tumor Transplantation :

The parent tumor line (Ehrlich Ascites Carcinoma) was supplied through the courtesy of Prof. Dr. M. M. El-Merzabani. The tumor cells were withdrawn from a 7 days old donor female Swiss Albino mice and diluted with physiological saline solution to give 12.5 $x10^6$ cells / ml. A 0.2 ml. was then injected intramuscularly in the right thigh. After 7 days of tumor transplantation. the animals with regular tumor were selected for this study .

The radioactive ^{14}C labelled misonidazole (ring - 2 - C^{14}), with specific activity of 3.8 μ Ci/mg , was used in this experiment. The purity of product had been checked by thin layer chromatography using butanol - water - acetic acid (120 : 50 : 30). One spot with R_f 0.8 was obtained which corresponded to misonidazole. The drug was emulsified in 0.1% w/v of Tween 80 and diluted to the appropriate concentration with isotonic phosphate buffer (pH 7.4).

Liposomal preparation:

In order to study the effect of liposomal drug encapsulation on the distribution pattern of the encapsulated drug, in different organs and serum, three different lipid compositions were used for both multilamellar and unilamellar types:

A) Neutral liposomes were prepared from dipalmitoyl phosphatidylcholine and cholesterol in the molar ratio of 7 : 3.

B) Positively charged liposomes were prepared from dipalmitoyl phosphatidylcholine, cholesterol and stearylamine in the molar ratios of 7:2:1.

C) Negatively charged liposomes were prepared from dipalmitoyl Phosphatidyl-choline, cholesterol and dicety phosphate in the molar ratios of 7 : 2 : 1.

Preparation of multilamellar liposomes :

Multilamellar liposomes were prepared by hand shaking method as described by Bangham et al. [42]. The lipids in chloroform were deposited as a thin film in a round - bottom flask by reduced pressure on a rotary evaporator. The final traces of chloroform was completely removed with a stream of nitrogen gas and subsequent evacuation at 25°C. The dried thin lipid film was hydrated with the appropriate amount of isotonic phosphate buffer solution (pH 7.4) containing the drug. The suspension was shaken gently by hand for about 1 hour under nitrogen gas at 25°C. This step was followed by vigorous shaking for 30 second, allowing to stand for 30 second, and then repeating ten times. The total liposomal lipid concentration was 60 μ mol/ml and the liposomes were adjusted to 12 μ mol / ml by dilution in the same buffer prior to extrusion.

Preparation of small unilamellar liposomes:

Small unilamellar liposomes were prepared from the previously formed multilamellar liposomes by

sonication. Liposomes were sonicated in a bath - type sonicator at 20°C for about 30 minutes (60 seconds sonication followed by 30 seconds cooling on ice - water mixture). Clarification of the turbid suspension indicates the conversion of multilamellar liposomes to small unilamellar liposome [43].

Sequential extrusion of liposomes:

The pressure extrusion method allows the sequential passage of preformed liposmes through membranes of decreasing pore diameter.

A homogeneous liposomal preparation with controlled particle size distribution was obtained by sequential extrusion through polycarbonate membranes. The liposomal size of these extrusions could be designated by the smallest membrane size through which the suspension was extruded [44].

A. Extrusion of multilamellar liposomes:

The multilamellar liposomal preparations were sequentially extruded through polycarbonate membrane filters with 3.0, 2.0, 1.0, and 0.8 μm pore size. The process was accomplished at a relatively low pressure (approximately 5 kg/cm^2) in 25 mm membrane holder.

B. Extrusion of unilamellar liposomes:

The unilamellar liposomal preparations were sequentially extruded through 0.6, 0.1 and 0.08 μm pore size polycarbonate membranes at a pressure of approximately 10 kg/cm^2.

Determination of drug encapsulation efficiency of different liposomal formutations:

Encapsulated drug was separated from unencapsulated (free) drug by centrifugation at 8,000 rpm for 15 minutes at 4°C. The supernatant was carefully decanted, for separation of the unencapsulated drug, and liposomes were resuspended gently in the same buffer used previously. This procedure was repeated to ensure complete removal of free drug. The supernatants from each process were collected and assayed spectrophotometrically [36] for determination of free drug concentration. The percentage of drug encapsulation within liposomes was calculated from the following equation :

$$\frac{\text{Amount of drug encapsulated}}{\text{Total amount of drug}} \times 100$$

Distribution studies of ^{14}C - labelled misonidazole in tumor-bearing mice :

The animals were divided into the following groups:

1- Mice intraperitoneally injected with ^{14}C - labelled misonidazole.
2- Mice intraperitoneally injected with small unilamellar liposomally* encapsulated drug.
3- Mice intraperitoneally injected with multilamellar liposomally encapsulated drug.
4- Both multilamellar and small unilamellar liposomal types of positive and negative surface charge were also injected to other groups of animals.

After intraperitoneal injection of the drug (1 mg / g body weight) the animals were sacrificed at 0.20, 0.30, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 24.0 and 48.0 hours. Blood samples were rapidly drawn from the jugular vein and serum was separated. Also, Samples from the tumor tissues and some selected organs (stomach, intestine, urinary bladder, spleen, muscle, brain, liver, kidney, heart and lung) were dissected out. Specimens were accurately weighed into scintillator counting vials and two ml of Hyamine hydroxide were added to each sample. The vials were tightly closed and shaken occasionally in a shaking water bath at 60°C till complete dissolution.

One ml of methanol was then added to each vial followed by 14 ml of scintillator solution having the following composition: 1000 ml of toluene containing 4 g PPO (toluene- 2, 5 - diphenyl oxazole) and 50 mg POPOP (1,4 - bis-(5-phenyl - oxazol-2-yl)-benzene).

Radioactivity measurements of samples were carried out using liquid scintillation counter (SC-722 automatic dual scalar/spectrophotometer.ICN Pharmaceutical, Belgium). The counts per minute per gram weight tissue or serum were corrected for quenching using internal standard [36].

Analysis of the data:

Each experiment was repeated four times. P values < 0.05 were considered significant. The data were analyzed statistically using the Student's t - test.

RESULTS AND DISCUSSION

The encapsulation efficiency of the drug in liposomes was studied using different liposomal lipid composition and preparation techniques. Preliminary experiment indicated that the encapsulation efficiency for labelled and unlabelled drug was the same .The data present in Table 1 clearly showed that multilamellar liposomes provided higher encapsulation efficiency than small unilamellar type.This observation could be related to the size and the mean number of bilayers of the liposomes where

* No sign of the net charge (prevailing at the liposomal surface) means neutral type.

different types of liposomes had comparable size and lamellarity.

On the other hand, regarding the liposomal surface charge for both types of preparations, the presence of charged lipids will tend to increase the encapsulation efficiency of the drug. However, negatively charged liposomes provided higher

Table (1): Effect of liposomal types and surface charge on the encapsulation of mesonidazole. Multilamellar and small unilamellar liposomes were prepared to contain 12 mol lipid/ml aqeous phase

Liposomal surface charge	% Encapsulation	
	Multilamellar liposomes	Small unilamella liposomes
1-Positively charged.	60.1 (0.5)*	29.8 (0.6)
2-Negatively charged.	78.7 (0.9)	53.6 (0.7)
3- Neutral	34.5 (0.6)	23.2 (0.9)

* The values between parentheses represent the standard error of the mean (n = 4).

encapsulation efficiency of the drug as compared to positively charged ones. This could be explained on the consideration that increasing the charge density will tend to increase the spacing between the bilayers by increasing the electrostatic repulsive forces. Furthermore, it was possible that the physicochemical properties of both the encapsulated drug and lipid composition will tend to maximize the encapsulation efficiency in presence of negatively charged lipid .

The distribution pattern of ^{14}C - labelled misonidazole in tumor and normal tissues of mouse for free and encapsulated liposomal forms was evaluated. Both multilamellar and small unilamellar types of neutral, positive and negative surface charge were studied. Measurements were performed at 0.20, 0.30, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 24.0 and 48 hours after administration of various formulations of the drug. Data were expressed as counts per minute per gram weight tissue. The results were graphically represented in Figures 1, 2 and 3. The results were statistically interpreted in terms of area under the curve, maximum concentration, time to reach maximum concentration and half-time ($t_{1/2}$)for clearance. The area under the curve was calculated using the trapezoidal rule. Values of these parameters were calculated and summarized in Table 2, 3 and 4.

General, for both normal and tumor tissues, the results showed that the drug was rapidly distributed throughout the selected various organs of the mice within 12 minutes. Also, it was clearly observed that the distribution pattern of the drug was variable with different formulations. Statistical analysis of the data generally indicated that , the liposamal drug encapsulated forms produced a greater influence on these parameters compared with the drug free form.

For normal tissues and serum (Figures 1,2 and Table 2,3), it was found that the uptake of liposomally entrapped drug into different organs and serum was significantly ($P< 0.05$) lower than that of free drug. Also, the half-time ($t_{1/2}$) for clearance of liposomally entrapped drug in these organs and serum was considerably longer than that of free drug. In addition, concerning the liposomal types, the results generally indicated that small unilamellar liposomes provided significantly ($P < 0.05$) higher drug uptake than multilamellar type. Moreover, the half-time ($t_{1/2}$) for clearance was significantly ($P< 0.05$) much longer with multilamellar liposomes than that observed with unilamellar type. Thus, depending on the drug formulation type, the uptake of the drug into normal organs and serum was found to be variable and could be arranged in the following descending order: drug free form > small unilamellar liposomally encapsulated drug > multilamellar liposomally encapsulated drug. On the other hand, comparing the different formulations, the half-time ($t_{1/2}$) for clearance of the drug could be arranged in the following descending order: multilamellar liposomal form > small unilamellar liposmal form > drug free form.

On the contrary, for tumor tissues, the data clearly demonstrated that the presence of tumor tissues resulted in a significantly ($P< 0.05$) increased uptake of the liposomally encapsulated drug into these tumor tissues compared with free drug (Figure 3,4 and Table 4). Also, the half-time ($t_{1/2}$) for clearance for tumor tissues was significantly ($P< 0.05$) much longer for liposomally encapsulated drug than that observed with free drug. Moreover, the effects of liposomal type and surface charge on the distribution pattern and clearance rate of the encapsulated drug in tumor tissues were investigated :

A. Depending on the liposomal type:

The results clearly showed that multilamellar liposomes provided significantly ($P< 0.05$) higher tumor uptake of the drug than small unilameller type. Also, the half-time ($t_{1/2}$) for clearance was significantly ($P< 0.05$) much longer for multilamellar liposomes than that observed with unilamellar type. This observation was most likely to be dependent on the liposomal size and number of bilayers or lamellarity, where it acts as a reservoir for prolonged release of the drug.

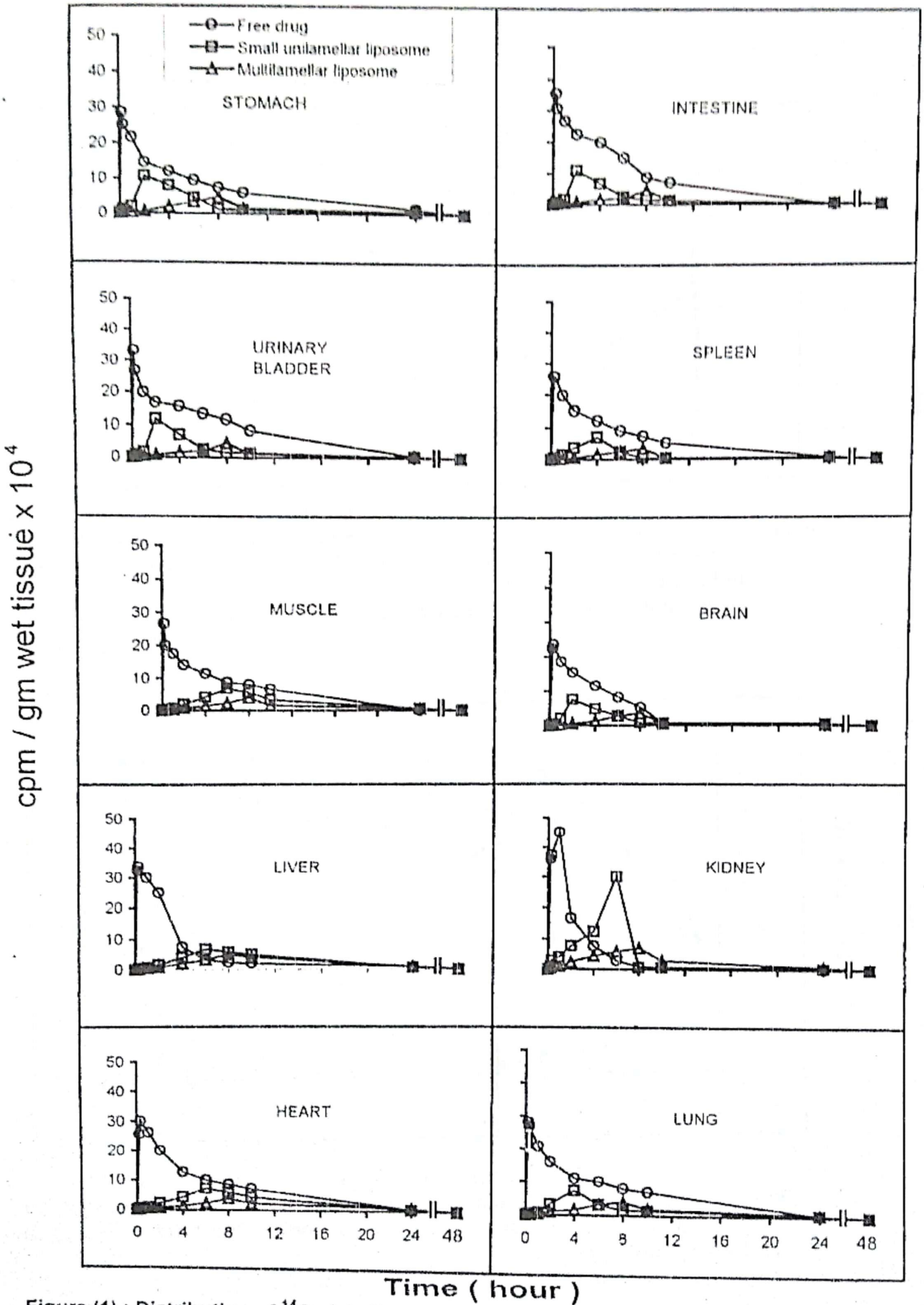

Figure (1) : Distribution of ^{14}C - labelled misonidazole in some selected organs of tumor-bearing mice following intraperitoneal administration of the drug. Comparison of free and liposomally entrapped drug.
Each point represents the mean value from four mice.

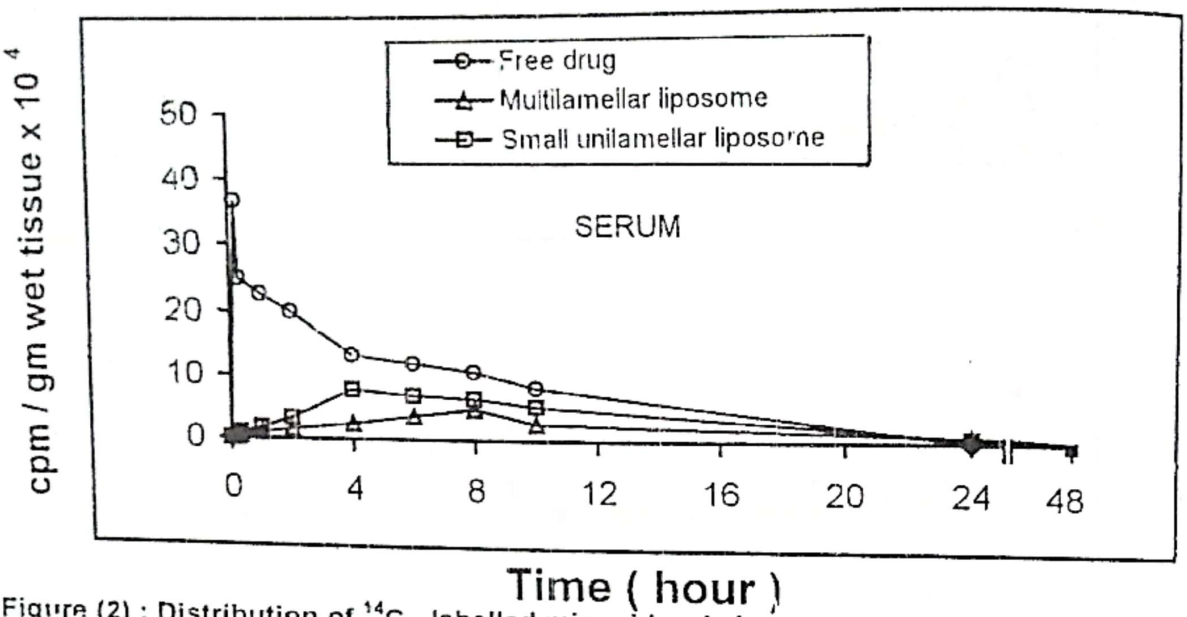

Figure (2) : Distribution of ^{14}C - labelled misonidazole in serum of tumor-bearing mice following intraperitoneal administration of the drug. Comparison of free and liposomally entrapped drug.
Each point represents the mean value from four mice.

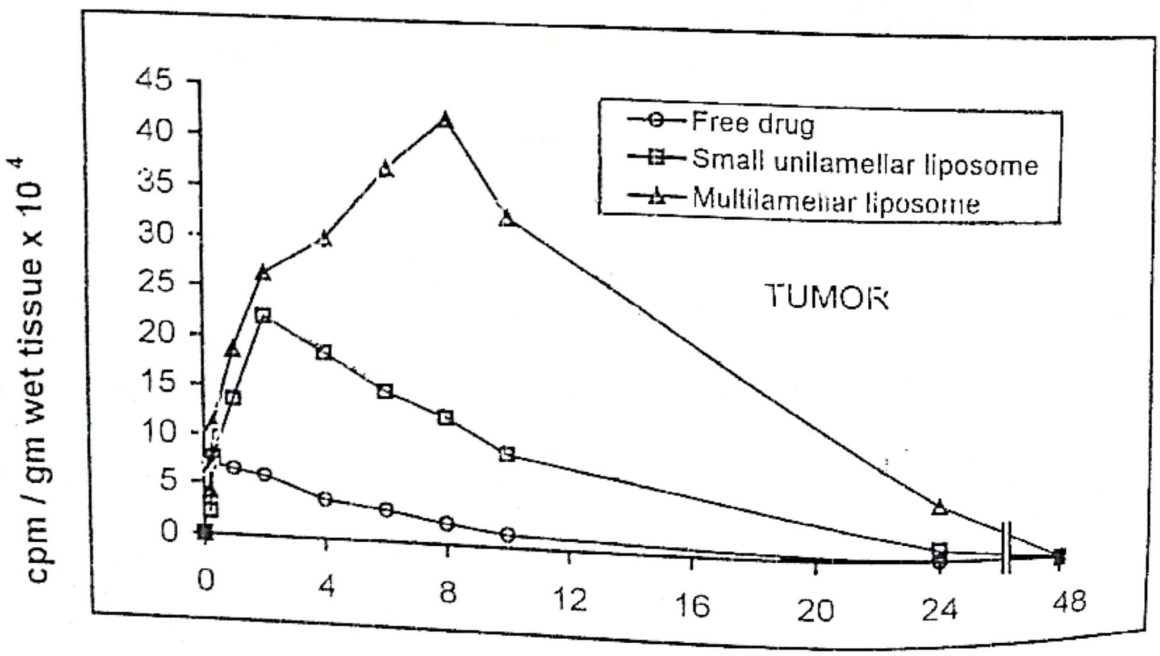

Figure (3) : Distribution of ^{14}C - labelled misonidazole in tumor tissues of tumor-bearing mice following intraperitoneal administration of the drug. Comparison of free and liposomally entrapped drug.
Each point represents the mean value from four mice.

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Table (2): Values for area under the curve , maximum concentration , time to reach maximum concentration and Half – time (T½) for clearance of 14C – labelled misonidazole in free and liposomal formulations for some selected organs .

Drug formulations	Organs	Area under the curve (cpm/gm hr. x104)	Maximum concentration*	Time to reach maximum concentration (hr.)	Half - time (T½) for clearance (hr.)
Free drug	Stomach	188.80	28.50	0.20	2.30
Small unilamellar liposomes		64.24	10.70	2.00	5.50
Multilamellar liposomes		48.13	4.66	8.00	9.30
Free drug	Intestine	217.90	35.50	0.20	2.16
Small unilamellar liposomes		54.54	11.00	2.00	5.00
Multilamellar liposomes		32.58	4.50	8.00	10.20
Free drug	Urinary bladder	220.54	33.10	0.20	4.15
Small unilamellar liposomes		49.98	11.70	2.00	5.00
Multilamellar liposomes		32.74	4.50	8.00	9.50
Free drug	Spleen	163.93	25.80	0.20	3.90
Small unilamellar liposomes		38.81	7.00	4.00	5.50
Multilamellar liposomes		27.88	3.40	8.00	9.00
Free drug	Muscle	157.35	20.00	0.30	3.00
Small unilamellar liposomes		72.16	6.66	6.00	9.80
Multilamellar liposomes		38.65	3.80	8.00	10.00
Free drug	Brain	111.98	23.50	0.30	3.80
Small unilamellar liposomes		44.27	7.33	2.00	5.90
Multilamellar liposomes		26.07	3.33	8.00	9.50
Free drug	Liver	132.62	33.57	0.30	3.00
Small unilamellar liposomes		82.03	6.66	6.00	16.00
Multilamellar liposomes		63.15	5.00	8.00	21.50
Free drug	Kidney	114.79	45.00	1.00	2.00
Small unilamellar liposomes		106.64	30.00	6.00	7.90
Multilamellar liposomes		83.04	7.00	8.00	10.00
Free drug	Heart	201.90	30.00	0.30	3.80
Small unilamellar liposomes		85.07	7.20	6.00	9.50
Multilamellar liposomes		55.31	3.90	8.00	21.00
Free drug	Lung	178.32	28.00	0.20	3.00
Small unilamellar liposomes		51.64	7.33	4.00	6.50
Multilamellar liposomes		46.49	4.00	8.00	10.20

* Expressed as concentration of radioactivity in cpm / gm tissue x10 4.

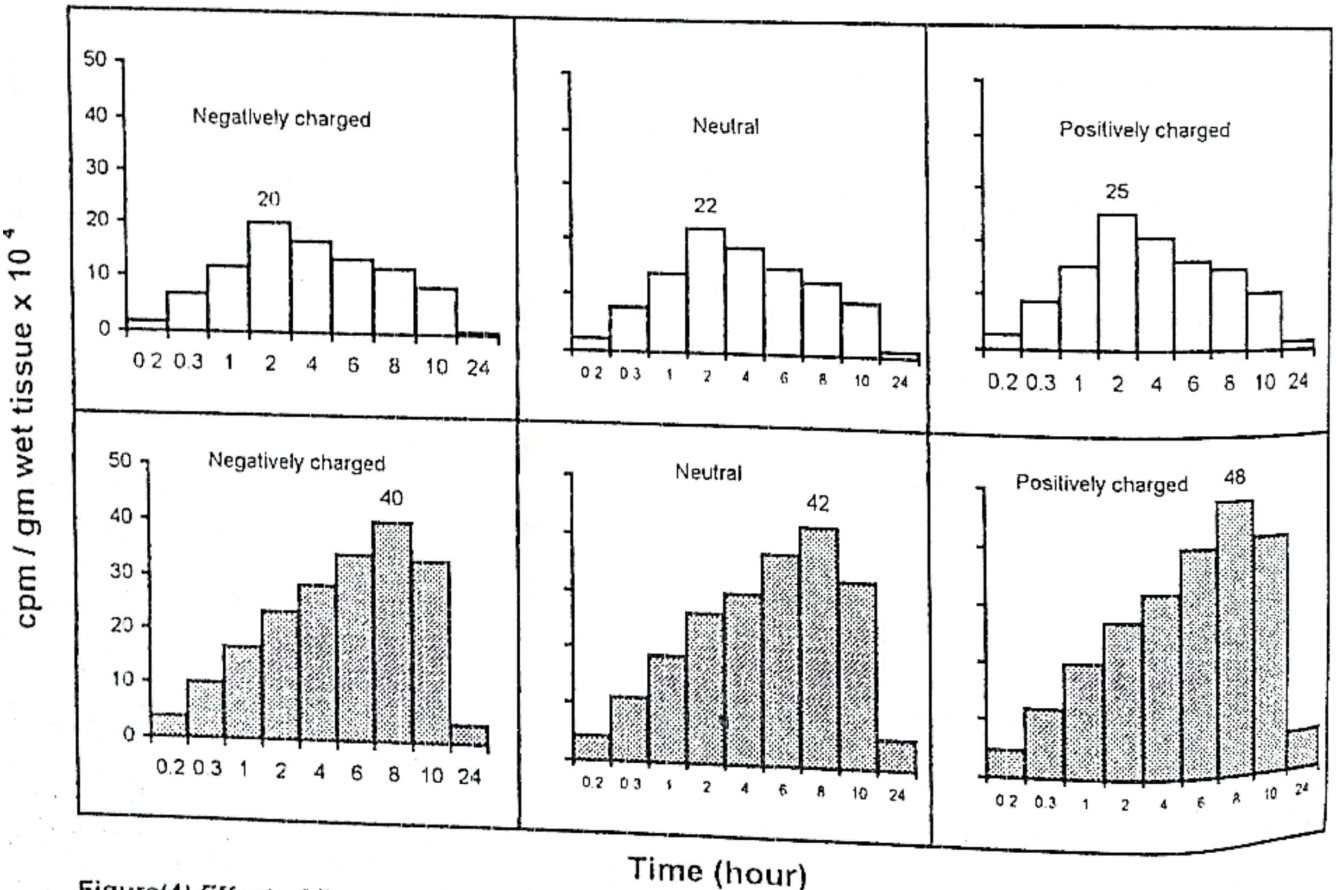
Table (3): Values for area under the curve , maximum concentration , time to reach maximum concentration and Half - time (T½) for clearance of 14C – labelled misonidazole in free and liposomal formulations for serum.

Drug formulations	Area under the curve (cpm/gm hr. $x10^4$)	Maximum concentration*	Time to reach maximum concentration (hr.)	Half - time ($T_{½}$) for clearance (hr.)
Free drug	203.42	36.60	0.20	3.20
Small unilamellar liposomes	104.13	7.80	4.00	16.10
Multilamellar liposomes	63.23	4.90	8.00	17.90

Table (4): Values for area under the curve , maximum concentration , time to reach maximum concentration and Half -time (T½) for clearance of 14C – labelled misonidazole in free and liposomal formulations for tumor tissues.

Drug formulations	Area under the curve (cpm/gm hr.x10^4)	Maximum concentration*	Time to reach maximum concentration (hr.)	Half life time ($T_{½}$) for clearance (hr.)
A- Free drug	45.66	7.00	0.20	6.30
B-Small unilamellar liposomes:				
1- Positively charged	268.12	25.00	2.00	11.70
2- Negatively charged	203.74	20.00	2.00	9.10
3- Neutral	233.82	22.00	2.00	10.20
C- Multilamellar liposomes:				
1- Positively charged	776.26	48.00	8.00	17.90
2- Negatively charged	565.16	40.00	8.00	15.80
3- Neutral	639.90	42.00	8.00	16.20

* Expressed as concentration of radioactivity in cpm / gm tissue x10 4.



Figure(4) Effect of liposomal types and surface charge on the distribution pattern of ^{14}C-labelled misonidazol in tumor tissues following intraperitoneal administration of the drug. Each point represents the mean value from four mice.
□ small unilamellar liposomes and ▨ multilamellar liposomes.

Each point represents the mean value from four mice.

Therefore, it could be suggested that the enhanced tumor uptake of the drug, using multilamellar liposomes as a carrier, may be attributed to the increased drug loading. Thus, liposomes may act as a slow - release depot with the establishment of a highlocalized concentration of the drug and maintaing its residence at the target cell.

As mentioned previously, all tissues take up the liposomally entrapped drug to some extent. Tumor tissues do also, but the uptake was significantly ($P< 0.05$) higher than that of normal tissues and the clearance rate was delayed. The most reasonable explanation for this is that liposomes are acting as a type of depot system, protecting the drug from deactivation while slowly releasing it into circulation for a prolonged period.

Prolonged tumor tissue retention of drug that is entrapped in liposomes compared to free drug may be attributed to the inherent lipophilicity of the liposomal bilayers and tumor tissue. For instance, the major components of bilayer structure of these liposomes were phospholipids which are natural constituents of cell membranes. In addition to the phospholipids, cholesterol was incorporated into liposomes, which may help in controlling drug retention by restricting the mobility of phospholipid and closer packing of the lipid bilayers making them more rigid [23,30,33]. Therefore, it can be assumed that liposomal formulations provided higher drug loading or can increase its local concentration at tumor tissues. Also, it was cleared from the published data that many of the tissues that take up liposomes are rich in endocytotic cells that do clearly take up liposomes. Thus tissue trapping and / or uptake by endocytotic cells could explain the preferential uptake of the liposomal entrapped drug by tumor cells [13,27,29]

B. Depending on the liposomal surface charge:

For both types of liposomal preparations, the tumor uptake of the drug and also half-time ($t_{1/2}$) for clearance were greatest for positively charged liposomes ,less for neutral liposomes and least for negatively charged liposomes (Figure 4 and Table 4). These observations could be due to the physicochemical nature of both liposomal lipid composition and tumor cell. Therefore, it seems reasonable to assume that the initial interaction and binding affinity between the tumor cells and liposomal surface may be electrostatic adsorption. This suggestion was in reasonably good agreement with previous studies reporting that the extensive binding between positively charged liposomes and tumor tissues could be attributed to the electrostatic attraction of negatively charged surface of the tumor cells and positively charged liposomal surface [31,36]

The role of liposomal surface charge in determining the tissue selectivity has received attention to liposomal systems. For example, Gregoriadis and Neerunjun [45] observed that negatively - charged liposomes were cleared more rapidly from the circulation than were neutral or positively - charged liposomes. There was also strong evidence that tissue uptake of liposomes is dependent on surface charge characteristics [28,46]. Thus, the liposomal surface charge will influence the behavior of the encapsulated drug and its specific ability to interact activily with the biological environment and the pathological condition of target cells. Thus, on the basis of the above presented results, it could be concluded that the liposomasl type and surface charge plays an important role influencing the distribution pattern and also the clearance rate of the encapsulated drug. Furthermore, it was clearly observed that multilamellar liposomes with positive surface charge represented an optimal or most suitable formulation, as a selective delivery system, for modulating drug action in cancer therapy.

In this way, liposomes of the appropriate composition and size could incorporate the drug into various cell compartments and upon attachment to the target site the drug has to be released from its carrier (liposome) to exert its action. Therefore, it was proposed that the ideal carrier for antitumor drugs should bind to the surface of tumor cells and not react with any other normal cells. After binding to the tumor cell membrane it should be endocytosed and reach the lysosomal compartment. Moreover, it should be able to cross the anatomical barriers to the target site [25, 27].

In conclusion, it was obvious that liposomal drug encapsulation can increase the cellular uptake of the tumor tissues and the rate of clearance of the encapsulated drug was delayed. Furthermore, liposomal type and surface charge can potentially be modified in a variety of ways to satisfy the needs of the formulation for targeting the drug to the selected site of action. Accordingly, liposomes seems to be a promising approach, of many potential benefits, for controlling and sustaining drug release within a specific target tissues and at a rate regulated by alteration in the lipid composition and variations in the liposomal preparation techniques.

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استخدام الليبوزوم كحامل لتصويب عقار الميثونيدازول إلي الخلايا السرطانية

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تمت دراسة تاثير تحميل العقار داخل الليبوزوم على نمط توزيعه فى أنسجة الفأر السليمة وكذلك السرطانية. وباستخدام الميثونيدازول المرقم ك[14] أمكن تتبع محتوى النشاطالإشعاعى للعقار فى بعض الأعضاء المختارة والمصل فى فترات زمنية مختلفة. وتبعاً لنوع صياغة المستحضر فإن مدى امتصاص العقار من الأنسجة السليمة كان متغير وكان أعلاها فى حالة استخدام العقار الحر وأقلها فى حالة استخدام العقار المحمول داخل الليبوزوم. ومقارنة لنوعى الليبوزوم فإن العقار المحمول داخل الليبوزوم وحيد الطبقة كان له درجة امتصاص أعلى من المحمول داخل الليبوزوم المتعدد الطبقات.

وعلى العكس من ذلك ففى حالة الأنسجة السرطانية فإن درجة امتصاص العقار كانت عالية باستخدام العقار المحمول داخل الليبوزوم المتعدد الطبقات مقارنة بالعقار المحمول داخل الليبوزوم وحيد الطبقة. وكذلك كانت أقل درجة امتصاص فى حالة استخدام العقار الحر. وتبعاً لنوع الشحنة الموجودة على سطح الليبوزوم فإن العقار المحمول داخل الليبوزوم والذى عليه شحنة موجبه كان أعلى درجة امتصاص من العقار المحمول داخل الليبوزوم ذو الشحنة السالبة أو المتعادلة.

وبذلك يمكن استنتاج ان الليبوزوم المتعدد الطبقات ذو الشحنة الموجبة يمثل الصيغة المثلى لتنظيم فاعلية العقار فى علاج السرطان ولتصويب العقار إلى المكان المطلوب استهدافه.