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The Distribution of Doxorubicin in Mice Following Administration in Niosomes

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Abstract—Large multilamellar non-ionic surfactant vesicles (niosomes) with diameters of around 800–900 nm prepared from a C_{16} triglyceryl ether with and without cholesterol and containing doxorubicin (Adriamycin) were administered to S180 tumour-bearing NMRI mice by bolus injection. Although in-vitro drug release from cholesterol-containing niosomes is delayed, in-vivo there was little difference between the two preparations when plasma levels were compared. As previously observed, half-lives of the drug were prolonged compared with free solution profiles. Liver uptake was not significantly affected by niosome encapsulation of doxorubicin. There is minor accumulation of drug in the lung, perhaps because of aggregation of the vesicles and their physical entrapment. Tumour levels of drug were higher following administration of cholesterol-containing niosomes and this was reflected in the more effective reduction in tumour growth. Metabolism of doxorubicin is altered by niosomal administration, but more studies are required before the significance of the metabolic data can be assessed.

The anthracycline antibiotic doxorubicin (Adriamycin; DOX) exhibits a broad spectrum of antitumour activity against leukaemias and solid tumours of both human and animal origin, many of which are unresponsive or respond poorly to other drugs. DOX is believed to exert its cytotoxic effect by acting as an intercalating agent with nuclear DNA, and rapidly inhibiting nucleic acid synthesis. Such a process, which is dependent on the rate and extent of the drug's accumulation into cells, may be limited in solid or other poorly perfused tumours.

Its clinical use is ultimately limited by a dose-dependent, irreversible cardiotoxic effect, manifested in the form of refractory congestive heart failure. Liposomal encapsulation of the drug has been shown to reduce the severity of cardiotoxicity in beagles and mice, whilst antitumour activity remains unaltered (Gabizon et al 1982).

Liposomal DOX has a more prolonged half-life and displays generally reduced toxicity, while maintaining, or having enhanced, cytotoxic activity in experimental animals (Olson et al 1982; Gabizon et al 1982; Rahman et al 1985). However treatment by these or similar carrier systems may be limited by accumulation of the carrier within the reticuloendothelial system. Such localized drug accumulation has, however, been exploited in treatment of animal tumours known to metastasize to the liver and spleen and in parasitic infestations of the liver (Alving 1983).

Niosomes are unilamellar or multilamellar vesicles formed from synthetic non-ionic surfactants of the alkyl or dialkyl polyglycerol ether class, offering an alternative to liposomes as drug carriers. Hydration of a film of a mixture of single-alkyl chain, non-ionic surfactant (Vanlerberghe et al 1972) and cholesterol leads to the formation of vesicular systems, termed niosomes (Vanlerberghe et al 1978; Baillie et al 1985). Cholesterol-free niosomes may also be produced. Niosomes can entrap solutes in a manner analogous to liposomes, are stable in-vitro, and can increase the stability of entrapped drug (Rogerson et al 1987). We have shown (Azmin et al 1985, 1986) that niosomes alter the pharmacokinetic profile, organ distribution and metabolism of entrapped methotrexate in mice. In these earlier studies small niosomes of around 120 nm diameter were employed.

In the present report the distribution of doxorubicin administered in larger vesicles (c 800–900 nm diameter) is compared with that following administration of an equivalent dose of free drug. The use of two niosome formulations allowed the influence of the cholesterol content of niosomes to be assessed. The work described is part of a larger programme which is concerned with the administration and activity of the drug in colloidal systems (Willmott et al 1985a,b). Increased antitumour effects observed after its administration directly into tumours in albumin microspheres have been ascribed (Willmott & Cummings 1987) to induction of DOX metabolism via reductive pathways. Levels of free drug are of importance in determining tissue penetration, while metabolite levels can dictate both toxicity and activity inside tissues. We have determined levels of DOX in six tissues, and observed the correlation between tumour drug concentration and the rate of growth of sarcoma S180. Metabolite levels were determined in some tissues.

Materials and Methods

Surfactant I, a C_{16} monoalkyl glycerol ether with an average of three glycerol units (Baillie et al 1985), was a gift from L'Oreal, France and was used as received. All solvents used were of HPLC reagent grade (Fisons Scientific, Loughborough, UK).

Pure doxorubicin HCl (Adriamycin HCl) and Adriamycinol HCl were gifts from Dr S. Penco (Farmitalia, Milan, Italy), daunorubicin was purchased from May & Baker (Dagenham, UK) and cholesterol from Sigma (Dorset, UK). 7-Deoxy-aglycones of DOX were synthesized as previously reported for validation of the HPLC assay (Cummings et al 1984).

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Preparation of niosomes

Niosomes were prepared as described by Baillie et al (1985). 150 μmol of surfactant I was dissolved in diethyl ether (10 mL) in a 50 mL round-bottomed flask. The ether was removed at 50°C under reduced pressure on a rotary evaporator to form a thin film on the flask wall which was then hydrated at 50°C for 15 min with gentle agitation with an aqueous solution of DOX (5 mg mL^{-1}, 3 mL). To prepare niosomes containing cholesterol this process was repeated using a 50:50 molar mixture of I:cholesterol. The niosome suspension in both cases was centrifuged at 10 000 *g* (1 h, 4°C) the supernatant discarded and the pellet first washed thoroughly with 60 mM phosphate buffer, pH 7·2, then resuspended in buffer (5 mL) to give a final DOX concentration of 1 mg mL^{-1}. The size of the niosomes as assessed by photon correlation spectroscopy, was 850 ± 150 nm.

Animal studies

Male, NMRI mice (25–30 g), about 3 months of age, from an inbred colony, were used. The S180 tumour was maintained by serial subcutaneous passage of tissue fragments in male NMRI mice.

Doxorubicin was administered as a bolus injection, via the tail vein, at a dose of 5 mg kg^{-1}. Animals (n = 3) were killed 10, 30 min, 1, 2, 4, 7, 16 and 48 h after injection, and tissues excised, washed in phosphate-buffered saline (PBS) and frozen to −70°C until use. The drug was extracted from tissues by the method of Cummings & Willmott (1985) which involved tissue homogenization followed by treatment with $AgNO_3$ solution (33% w/v) to remove DOX intercalated to DNA, then extraction from 5 vol chloroform–propan-2-ol (2:1), while serum samples involved only the solvent extraction step. The HPLC method used to determine the drug and metabolite levels has been described by Cummings et al (1984).

The niosomes prepared as described were not sterilized and had been tested for neither pyrogen content nor immunogenicity. Following administration of DOX-loaded niosomes, of both types, to over 70 mice no fatalities were encountered that could be attributed to the preparation.

On the third day following subcutaneous passage of S180 tumours (0·2 g), mice (n = 3) were administered a dose of 5 mg kg^{-1} DOX in free solution, or in niosomes, with a control group treated with PBS. Tumour mass was estimated from diameter measurements, via a calibration graph, for 16 days following drug administration.

Results and Discussion

Serum

Cummings et al (1984) found that doxorubicin was rapidly distributed from the serum following i.v. injection and that there was a concomitant rapid attainment of equilibrium in liver and heart tissue. This was followed by a subsequent slow-phase plasma drug clearance. A similar pattern of drug elimination was observed in the present study. However, when the drug was administered in niosomal form with and without cholesterol, plasma levels of it were higher for almost the entire course of study (Fig. 1), than when the solution was given, confirming the sustained release characteristics of niosomes. There was no significant difference between the two niosome types, despite the increased permeability of the cholesterol-free niosomes as demonstrated by the increased in-vitro efflux, in buffer and plasma, of both carboxyfluorescein (Baillie et al 1985; Hume 1987) and DOX (Rogerson et al 1987). However, the low drug levels obtained 48 h after administration in cholesterol-free vesicles are most likely to be the result of this more rapid drug release.

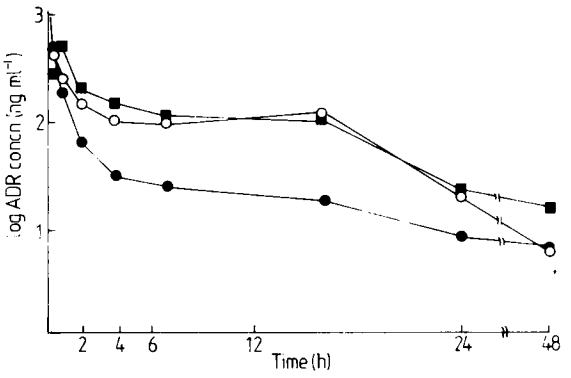

FIG. 1. Serum concentrations of doxorubicin as a function of time after administration of 5 mg kg^{-1} as a bolus injection into the tail vein of male NMRI mice. (●) solution of drug; (■) niosomes prepared from surfactant I alone; and (○) niosomes prepared from a 50:50 mixture of cholesterol and surfactant I.

Liver

Contrary to our findings with methotrexate-loaded niosomes (Azmin et al 1985), our present results suggest that little accumulation of DOX-containing niosomes occurs in the liver (Fig. 2). The cholesterol-containing niosomes provided higher tissue levels, which may be a consequence of the slower release of entrapped drug from these vesicles, causing intact niosomes of this type within the hepatic capillaries to be likely to contain a greater quantity of entrapped drug. The lack of excessive liver accumulation after niosomal DOX has been confirmed by us in another animal model (Kerr et al unpublished).

Intravenously administered liposomes generally become associated with organs of the reticuloendothelial system, mainly the liver and spleen (Forssen & Tokes 1979) resulting in very high tissue concentrations from drug-loaded liposomes (Kimelberg et al 1976; Abra & Hunt 1981). In

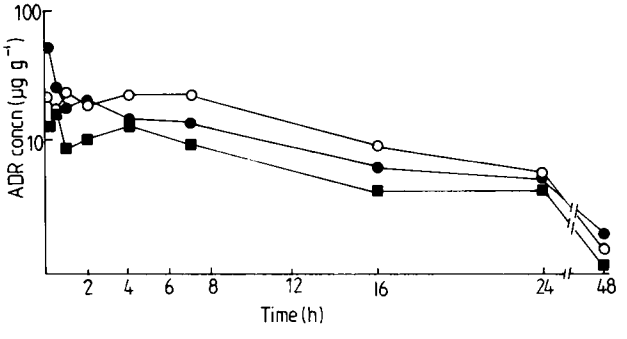

FIG. 2. Concentrations of doxorubicin in the liver of male NMRI mice following injection of 5 mg kg^{-1} as solution (●) and niosomes (■, ○). Symbols as in Fig. 1.

addition, this rapid partitioning of drug from the circulation is often accompanied by a marked reduction in pharmacological activity, as a result of accumulation at a major site of metabolism. However, Rahman et al (1985) have also found that administration of DOX-loaded cardiolipin liposomes to mice did not increase liver levels of the drug. The reasons for the apparent differences in liver accumulation have to be resolved, but are likely to revolve around the size, charge and surface characteristics of the vesicles

Previous work in mice, has suggested that niosomes may suffer uptake by the reticuloendothelial system. Methotrexate (Azmin et al 1985) and sodium stibogluconate (Baillie et al 1986) were both found to accumulate in the liver following administration in niosomes. The lack of elevation of liver DOX levels noted in this study must be a consequence of the drug and its effect on the carrier or on the carrier itself. In our work with methotrexate smaller (ca 120 nm diameter) negatively charged vesicles prepared with dicetylphosphate (DCP) were employed. The systems used in his work are larger (ca 800 nm), have no DCP and are neutral or positively charged. It might be argued that the absence of accumulation in the liver supports the contention that the sustained plasma levels of drug from niosomes results from slow release from circulating rather than trapped vesicles, but we have no definitive evidence to support this.

Heart

Cardiac tissue concentration profiles for each of the three systems were similar over the course of the study, but cholesterol-free vesicles were generally the more successful of the two drug carriers at reducing cardiac levels (Fig. 3). Clinical use of doxorubicin in man can be limited by cardiotoxicity in the form of refractory congestive heart failure characterized by cytoplasmic vacuolization and myofibrillar loss.

Gabizon et al (1982) reported that DOX-loaded cardiolipin liposomes, which were relatively permeable to entrapped drug, did not reduce cardiac levels in mice, while the less permeable vesicles used by Rahman et al (1985) reduced cardiotoxocity. Tissue uptake of liposomes is believed to be mediated by membrane fusion or endocytosis (Poste & Papahadjopoulos 1976) and, as cardiac tissue displays a low level of phagocytotic activity (Trouet et al 1972) this would result in a lower drug uptake. The results mirror those obtained in the liver. This suggests that the drug was retained to an extent sufficient to override the phenomenon of poor vesicular phagocytosis.

The interpretation of results of drug delivery in carrier systems is not simple because we cannot distinguish between free and encapsulated drug in the tissue samples. We measure total drug levels. More rapid drug loss from leaky niosomes would provide a greater quantity of drug in solution available to be taken up by passive diffusion or carrier-mediated transport (Skovsgaard 1978), but would leave less in the entrapped vesicles. Resolution of this problem awaits modelling of the processes involved. Gabizon et al (1982) has observed similar results with cardiolipin vesicles, while Forssen & Tokes (1979) reported that phosphatidylcholine liposomes significantly reduced drug concentrations in cardiac muscle.

Perhaps a more relevant indicator of cardiac toxicity is the presence of 7-deoxyaglycones of both doxorubicin and adriamycinol (see below).

Lungs

Levels of the drug in the lung are higher for the first 6 h after injection of niosomal drug compared with free drug, as shown in Fig. 4. It has been reported that DOX-loaded phosphatidylcholine liposomes have the capacity to raise lung drug concentrations by a factor of 7 (Gabizon et al 1982).

Drug-free niosomes produced by the technique described are approx 900 nm in diameter; in the presence of electrolyte, e.g. NaCl, these undergo rapid and extensive aggregation and an approximately two-fold increase in apparent diameter reversible on sonication (Rogerson et al 1988). Although vesicles of such proportions would not be completely sieved out by the alveolar capillaries, retention of a substantial quantity could account for the observed increase in tissue levels and this might affect the availability of vesicles for entrapment in other organs. In addition to physical alveolar entrapment, drug levels may also be increased from niosome preparations due to the influence of the rapidly proliferating alveolar phagocytotic cells within the basement

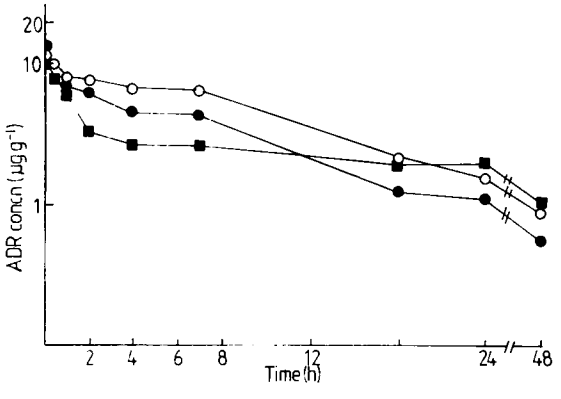

FIG. 3. Concentrations of doxorubicin in the heart of male NMRI mice after injection of 5 mg kg^{-1} as solution (●), or entrapped in niosomes (■, ○). Symbols as in Fig. 1.

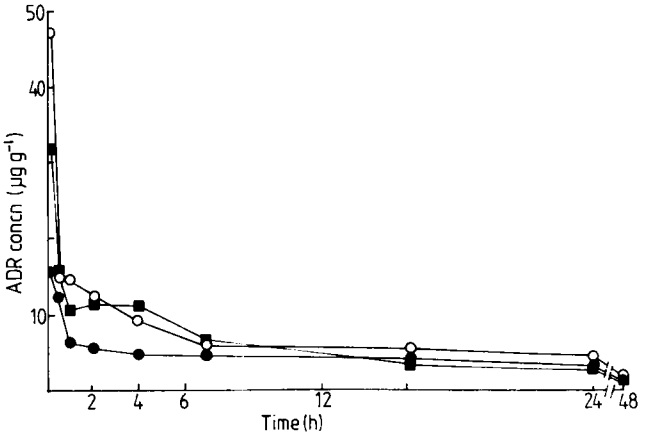

FIG. 4. Concentrations of doxorubicin in the mouse lung after tail vein injection of a bolus of 5 mg kg^{-1} as solution (●) or in niosomes (■, ○).

epithelium which, like other macrophages, will have the capacity to phagocytose the particle drug carriers from the circulation. Histological examination of lung sections taken 2 min after i.v. administration of drug-free niosomes revealed apparent accumulation of phagocytic cells within the alveolar membrane, which were not apparent in animals similarly treated with PBS.

Spleen

Drug concentrations in the spleen displayed characteristics similar to those of the liver, in that there was no significant difference between the three formulations, presumably as a result of the effects discussed above. Despite the fact the liposome preparations administered by i.v. injection extensively accumulated in the liver and spleen (Kimelberg et al 1976), it has been reported by Rahmann et al (1978) that actinomycin D levels in the spleen are not increased.

Tumour

The concentration of the drug in S180 sarcoma has been increased, even after a single injection. Doxorubicin profiles following administration of free and niosomal drug are shown in Fig. 5. After the initial peaks (30 min), levels of niosomal-DOX were slightly higher than following free drug administration, though only to a significant degree following administration in cholesterol containing niosomes. The higher tumour concentrations was reflected in an increased antitumour activity following a single 2·5 mg kg^{-1} i.v. bolus to S180-bearing mice (Fig. 6). Results suggested that administration of DOX-loaded, cholesterol-free niosomes increased the life-span of tumour-bearing mice and decreased the rate of proliferation of the sarcoma. However such a sarcoma may not be an appropriate model for the human tumours generally treated with the drug because of its lack of metastatic activity (Gabizon et al 1982).

Further work with multiple injections of the formulations is necessary. Gabizon et al (1982) have shown that multiple i.v. injection of DOX-loaded liposomes can induce a significantly greater antitumour effect than an equivalent dose of free drug in the treatment of J-6456 lymphoma, and Forssen & Tokes (1981) demonstrated a similar effect with doxorubicin against L-1210 and P-388 murine leukaemic models. Those workers, and others (Rahman et al 1985) have monitored mean survival time as a means of assessing antitumour activity.

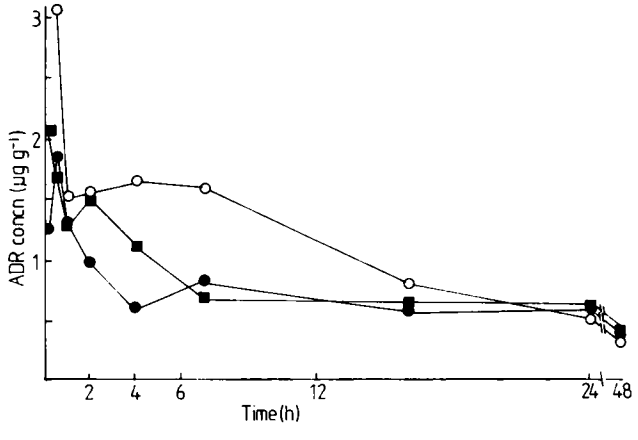

FIG. 5. Concentrations of doxorubicin in the S180 tumours in male NMRI mice as a function of time after injection via the tail vein of 5 mg kg^{-1} of drug. Symbols as in Fig. 1.

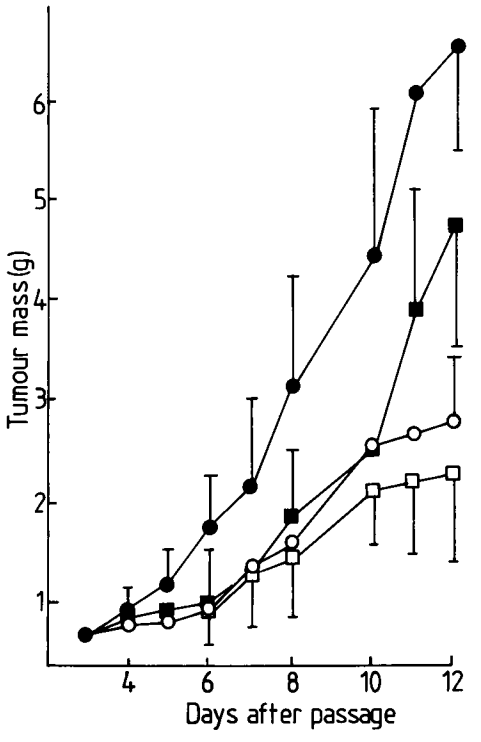

FIG. 6. The growth in the mass of implanted tumour as a function of time after injection of (●) control (PBS); (■) free solution of doxorubicin, 5 mg kg^{-1}, (○) drug in cholesterol-free niosomes, or in (□) niosomes containing 50 mol percent cholesterol.

Metabolisms of doxorubicin

Serum levels of doxorubicin 7-deoxyglycone (AOL-7-DONE) following administration of both free and niosomal drug are shown in Fig. 7. The levels of metabolite after free drug solution is administered intravenously fall rapidly over the first 4 h, and very low levels (< 0·5 ng mL^{-1}) persist for up to 24 h. The profile of AOL-7-DONE levels is altered by administration in both types of niosomes, t_{max} being shifted to longer times (> 2 h for the cholesterol-containing niosomes), and the AUCs being much greater in both cases. A similar trend has been observed following intratumoural injection of doxorubicin in albumin microspheres (Willmott & Cummings 1987), and possibly could reflect simply its slow release for metabolic breakdown. Administration of methotrexate in niosomes reduces the formation of its 7-hydroxy metabolite (Azmin et al 1986). The complexity of the metabolic profile of doxorubicin and of the activity-toxicity spectrum of its metabolites makes it difficult to speculate on the benefits to be gained, but it is clear that administration of the drug in carrier systems changes its metabolic behaviour. This must be taken into account in assessing the utility of these forms of administration.

Conclusions

Increased drug retention in the serum following administra-

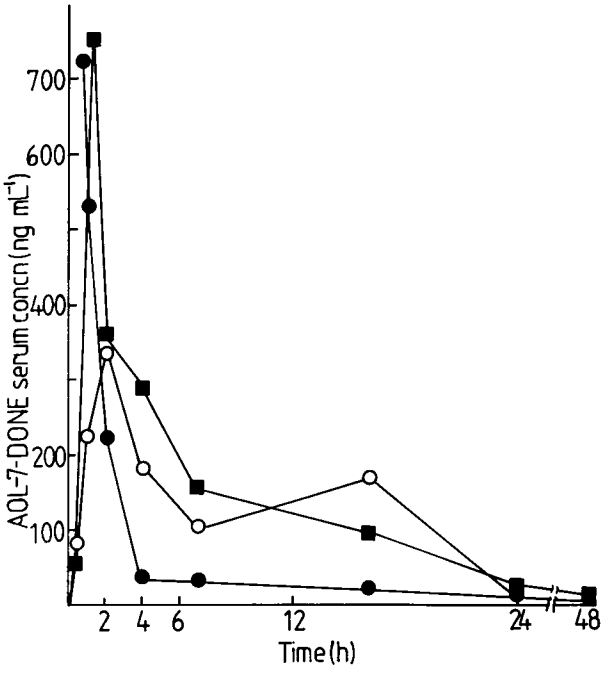

FIG. 7. Concentrations of one doxorubicin metabolite, adriamycinol-7 deoxyaglycone (AOL-7-DONE) in mouse serum after injection of (●) free drug in solution; (■) drug in cholesterol-free niosomes; and (○) drug in niosomes containing 50 mol percent cholesterol.

tion of doxorubicin in vesicular form results in a potential increase in the duration of action of the drug, particularly against well perfused tumours, by decreasing its partitioning to non-active sites or major organs of distribution (Weinstein 1984). In the light of our study there would perhaps be some potential benefit from administration of DOX-loaded niosomes in certain leukaemic disorders. The niosomes we used do not appear to accumulate in the liver and spleen, perhaps because a significant proportion is filtered out in the passage through the lung capillary network. It might be that the significance of cholesterol in niosomes goes beyond the effect it exerts on drug release, perhaps influencing phagocytosis and cellular uptake, although this cannot yet be proved. The raised doxorubicin levels in tumours after administration in cholesterol-containing niosomes is reflected in a marginally improved activity. We have demonstrated prolonged circulation of drug, altered metabolism, and maintenance of antitumour effect on delivery of drug in niosomes, but further work is required to establish appropriate dosage regimens to achieve tumour regression and to gauge the significance of changes in the metabolism of the drug before studies in the clinic.

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