Immunoliposome-mediated targeting of doxorubicin to human ovarian carcinoma in vitro and in vivo

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Summary This paper deals with the utility of immunoliposomes for the delivery of doxorubicin (DXR) to human ovarian carcinoma cells in vitro and in vivo. We aimed to investigate whether immunoliposome-mediated targeting of DXR to ovarian cancer cells translates in an enhanced anti-tumour effect compared with that of non-targeted DXR liposomes (lacking the specific antibody). Target cell binding and anti-tumour activity of DXR immunoliposomes were studied in vitro and in vivo (xenograft model of ovarian carcinoma). In vitro we observed that target cell binding and cell growth inhibition of DXR immunoliposomes is superior to that of non-targeted DXR-liposomes. However, in vivo, despite the efficient target cell binding and good anti-tumour response of DXR-immunoliposomes, no difference in anti-tumour effect, compared with non-targeted DXR-liposomes, could be determined. The results indicate that premature DXR leakage from immunoliposomes occurring before the actual target cell binding and subsequent DXR association with the tumour cells, explains why no significant differences in anti-tumour activity between DXR-immunoliposomes and non-targeted DXR-liposomes were observed in vivo.

Keywords: immunoliposome; doxorubicin; targeted drug delivery; ovarian cancer; monoclonal antibody

Ovarian cancer is associated with a high incidence and the highest mortality compared with other gynaecological malignancies. As ovarian carcinoma remains confined to the peritoneal cavity throughout most of its clinical course, this type of cancer is an attractive candidate for intraperitoneal (i.p.) chemotherapy (Straubinger et al., 1988; Markman, 1991; Nässander et al., 1992). Clinical pharmacokinetic studies have demonstrated that i.p. administration results in higher concentrations of drug at the site of disease, whereas systemic plasma concentrations, and therefore systemic toxicity, remain lower than after intravenous (i.v.) administration. Local anti-tumour activity after i.p. chemotherapy may therefore be expected to be higher with lower systemic toxicity.

Intraperitoneal administration of doxorubicin (DXR), a powerful broad-spectrum antineoplastic agent, is greatly hampered by local toxicity, in particular dose-limiting peritonitis. Therefore, investigators did not continue to use DXR for i.p. chemotherapy of ovarian carcinoma (Ozols et al., 1982; Deppe et al., 1985; Markman et al., 1989). Preclinical and clinical evidence shows that the local inflammatory reaction produced by DXR is reduced by encapsulation of DXR in liposomes (Forssen and Tokes, 1981; Delgado et al., 1989). In a phase I/II trial it was shown that DXR-liposomes can be administered i.p. with minimal peritonitis up to a three times higher dose per cycle than free DXR (Delgado et al., 1989). Even then, maximum tolerable dose was not reached with DXR-liposomes.

Earlier, we and others have shown that the use of antibodies to direct drugs encapsulated in liposomes to tumours creates an interesting possibility for increasing the specificity and efficacy of i.p. chemotherapy of tumours located in the peritoneal cavity (Straubinger et al., 1988; Singh et al., 1991; Nässander et al., 1992). To target the liposomes specifically to the tumour cells present in the peritoneal cavity, Fab' fragments of the monoclonal antibody OV-TL3, which is directed against the antigen OA3 present on over 90% of all human ovarian carcinomas, were coupled to the surface of the liposomes. When administered i.p., such OV-TL3-immunoliposomes bind rapidly and efficiently (more than 80% of the injected i.p. dose) to human ovarian cancer cells located in the peritoneal cavity of nude mice (Nässander et al., 1992).

The aim of this study was to investigate whether immunoliposome-mediated targeting of DXR to ovarian cancer cells translates into an enhanced anti-tumour effect compared with non-targeted DXR-liposomes (lacking the antibody). Target cell binding and anti-tumour activity of DXR-immunoliposomes were studied in vitro and in vivo. The i.p. growing NIH:OVCAR-3 tumour was chosen for in vivo evaluation as this model has many features in common with clinical disease, including development of abdominal disease, ascites formation and expression of the tumour-associated antigen OA3 (Hamilton et al., 1984; Poels et al., 1986; Moseley et al., 1988; Boerman et al., 1990). This is the first report describing the in vivo anti-tumour activity of DXR-immunoliposomes in a xenograft model of i.p. growing ovarian carcinoma.

Materials and methods

Materials

Fetal calf serum (FCS) was obtained from Boeknek Laboratories, Canada. RPMI-1640 medium supplemented with 25 mM Heps buffer and 1-glutamine were obtained from Gibco (Breda, The Netherlands). Dulbecco's modified Eagle's medium (DMEM) was supplied by ICN Flow (Zoetermeer, The Netherlands). F(ab') fragments of the monoclonal antibody OV-TL3 were donated by Centocor Europe BV (Leiden, The Netherlands). DXR was a gift from Pharmachemie (Haarlem, The Netherlands). Egg phosphatidylcholine (EPC), cholesterol (CHOL), trichloroacetic acid (TCA), dithiothreitol (DTT) and sulphorhodamine-B (SRB) were obtained from Sigma (St Louis, MO, USA). Egg-
phosphatidylglycerol (EPG) was a gift from Nattermann Phospholipid GmbH (Cologne, Germany). Phosphatidylethanolamine (PE) was obtained from Nutfield Nurseries Lipid Products (Nutfield, UK). Succinimidyl 4-(p-maleimidophenyl)butyrate was obtained from Pierce (Rockford, USA). [1a,2a(n)-3H]Cholesteryl oleyl ether, [U-14C]Sucrose and [14-1C]doxorubicin hydrochloride were obtained from Amersham (Buckinghamshire, UK). All other reagents were of analytical grade.

Monoclonal antibody

The murine monoclonal antibody OV-TL3 is directed against the OA3 antigen, present on over 90% of all human ovarian carcinomas (Poels et al., 1986; Boerman et al., 1990; Massuger et al., 1991). Fab′/F(ab′)2 fragments of OV-TL3 were incubated with 20 mM DTT in acetate buffer at pH 5.5 (100 mM sodium acetate, 63 mM sodium chloride, 1 mM EDTA) for at least 90 min at room temperature (Nässander et al., 1995). DTT was removed by applying the incubation mixture onto a Sephadex G-25M column (PD-10; Pharmacia, Woerden, The Netherlands). Elution occurred with acetate buffer pH 6.5 (100 mM sodium acetate, 40 mM sodium chloride, 1 mM EDTA, deoxygenated and flushed with nitrogen before use). Fab′ fragments appearing in the void volume were used immediately for covalent attachment to freshly prepared liposomes containing the anchor molecule N-[4-(p-maleimidophenyl)butyryl] phosphatidylethanolamine (MPB-PE).

Preparation of immunoliposomes

MPB-PE was synthesised, purified and analysed as described above (Martin and Papahadjopoulos, 1982; Nässander et al., 1995). MPB-PE was incorporated into the liposomal bilayers to allow covalent coupling of Fab′ fragments to the liposomal surface. The composition of the bilayer of the liposomes used was EPC:EPG:CHOL:MPB-PE at a molar ratio of 38.1:4.32:1.9. For cell binding and biodistribution experiments, traces of [3H]cholesteryl oleyl ether were added. A mixture of the appropriate amounts of lipids in chloroform was evaporated to dryness in a rotary evaporator at 35°C under reduced pressure. After flushing the lipid film with nitrogen for at least 20 min, the lipid film was hydrated in a 120 mM ammonium sulphate solution containing 1 mM desferal. After ten freeze--thaw cycles (freezing in liquid nitrogen at -196°C, thawing at 60°C), the resulting liposome dispersion was sequentially extruded through polycarbonate membrane filters with 0.6 µm and 0.2 µm pore size (Unipore, Biorad, Richmond, CA, USA) under nitrogen pressures up to 0.8 MPa, which resulted in a mean particle size of approximately 0.25 µm.

The liposomes were loaded with DXR by creating an ammonium sulphate gradient as described recently (Haran et al., 1993). After extrusion, the external medium of the liposomes was replaced by a glucose (0.2 M) – sodium chloride (37.5 mM) solution using ultracentrifugation (100 000 x g, 45 min). Then, liposomes were incubated with DXR for 30 min at 40°C (incubation conditions 15 µmol total lipid (TL) per ml; 1 mg DXR ml⁻¹; 1 mM desferal). Non-encapsulated DXR was removed using the cation exchange resin Dowex 50WX-4 (Storm et al., 1985). The liposomes were centrifuged (100 000 x g, 30 min) and the pellet was redispersed in acetate buffer pH 6.5. The freshly prepared DXR-liposomes containing the anchor molecule MPB-PE were mixed with freshly prepared Fab′ fragments (concentrations during incubation ranged from 6–12 µmol TL per ml and 0.25 – 0.35 mg Fab′ per ml respectively). The coupling reaction was carried out overnight at 4°C under constant rotation in nitrogen atmosphere.

Finally, the DXR-immunoliposomes were separated from un conjugated Fab′ fragments by ultracentrifugal sedimentation at 100 000 x g for 30 min. The pellet was resuspended and washed twice with Heps buffer (20 mM Heps, 149 mM sodium chloride, 1 mM EDTA, pH 7.4). MPB-PE-containing liposomes that were not incubated with Fab′ fragments are referred to as non-targeted liposomes throughout this paper. Liposome dispersions were stored at 2–8°C and used within 3 weeks after preparation.

Liposome characterisation

Lipid phosphate was determined by the colorimetric method of Fiske and Subbarow (1925). The amount of protein coupled to the liposomes was determined by the method of Wessel and Flügge (1984), with bovine serum albumin as standard. The amount of monoclonal antibody coupled to the liposomes was expressed as µg Fab′ per µmol TL. DXR was determined fluorimetrically after destruction of the liposomes with acidified ethanol (94% ethanol, 0.3 M hydrochloric acid; excitation wavelength 490 nm, emission wavelengths 591nm). Radioactivity of the liposomal dispersions was assayed with Hionic-Flour (Packard Instruments, Downers Grove, Illinois, USA) as scintillation mixture and counted in a Tri-Carb 1500 liquid scintillation counter (Packard Instruments). Mean particle size was determined by dynamic light scattering with a Malvern 4700 system using a 25 mW helium–neon laser and the Autoimage version 3.2 software (Malvern, Malvern, UK). For viscosity and refractive index values of pure water were used. As a measure of the particle size distribution of the dispersion, the system reports a polydispersity index. This index ranges from 0.0 for an entirely monodisperse up to 1.0 for a polydisperse dispersion.

Tumour cell line

The human ovarian cancer cell line NIH:OVCAR-3 originated from Dr TC Hamilton (Hamilton et al., 1983, National Cancer Institute, Bethesda, MD, USA). The NIH:OVCAR-3 cells were maintained in DMEM supplemented with heat-inactivated FCS (10%), glutamine (2 mM), penicillin (100 units ml⁻¹), streptomycin (100 µg ml⁻¹) and amphotericin B (0.26 µg ml⁻¹).

In vitro cell growth inhibition

In vitro cell growth inhibition was determined by the SRB assay which involves the measurement of cellular protein, using the dye sulforhodamine-B (SRB) (Rubinstein et al., 1990; Skehan et al., 1990). Briefly, ovarian tumour cells cultured as monolayers were treated with trypsin/EDTA (0.05%/0.02%) and washed with medium. Subsequently, cells (4 x 10⁴ ml⁻¹) were incubated in suspension for 30 min at 37°C with DXR-immunoliposomes, non-targeted DXR-liposomes or free DXR. After the incubation, unbound liposomes and remaining free DXR were removed by centrifugation (500 x g, 3 min). The cell pellet was washed twice and resuspended in culture medium. Then, 4 x 10⁴ cells per well were seeded in a flat bottom 96-well plate and cultured for 72 h at 37°C and 5% carbon dioxide. The cultures were fixed with 5% trichloroacetic acid (TCA) at 4°C for 1–2 h, washed with water and finally stained with 0.4% SRB dissolved in 1% acetic acid. After 20 min, the plates were washed with 1% acetic acid and air dried. The bound dye was dissolved in 10 mM Tris and the optical density was measured at 490 nm using a Biorad novapath microplate reader (Biorad Laboratories, Venendaal, The Netherlands). The cytotoxic activity of DXR is expressed as relative cell growth, which is defined as the degree of cell proliferation relative to that of non-treated cells (= 100%) and is presented as IC₅₀, i.e. the DXR concentration that induces 50% cell growth inhibition compared with non-treated cells.

Binding of DXR-immunoliposomes to ovarian cancer cells in vitro

In vitro cell binding of DXR-immunoliposomes was studied using DXR-liposomes containing 'H-labelled cholesteryl oleyl ether. OVCAR-3 cells were harvested as described above and...
incubated with the radiolabelled liposomes at 37°C for 30 or 90 min under the following conditions: 4 x 10^6 cells ml⁻¹, 0.88 ± 0.08 µmol TL ml⁻¹ and 0–14 µg DXR ml⁻¹. Unbound liposomes were separated from the cells by centrifugation (500 x g, 5 min). The cell pellet was washed twice with medium, mixed with 1 ml Soluene-350 (Packard) and digested at 40°C overnight. Radioactivity was measured as described above.

The amount of DXR associated with OVCAR-3 cells was studied by fluorescence analysis. OVCAR-3 cells (1 x 10⁶ cells ml⁻¹) were incubated in suspension at 37°C for 30 min with [³H]-cholesterol-labelled liposomes or non-targeted DXR-liposomes at a DXR concentration of 125 µg ml⁻¹. Unbound liposomes were separated from the cells by centrifugation (500 x g, 5 min). The cell pellet was washed twice with ice-cold medium, resuspended in 0.5 ml 0.05% paraformaldehyde in PBS and kept on ice until fluorescence was measured. Fluorescence analysis was performed using a single laser FACSscan, at a wavelength >650 nm (Becton and Dickinson Immunocytometry Systems, Mountain View, CA, USA).

Animals and tumour model
NMRI athymic nude mice (Ryagaard and Friis, 1974) were bred at Harlan CPB (HsdCpn:NMRI-rcw, Zeist, The Netherlands). The animals were housed throughout the experiment under specified pathogen-free conditions in sterile filter-top cages. They received sterile standard food (SRM-A, Hope Farms, Woerden, The Netherlands) and acidified sterile water ad libitum. At the start of the experiment the animals were 5–10 weeks old.

The tumour cell line NIH:OVCAR-3 was propagated in vitro cell growth inhibition

OV-TL3-immunoliposomes containing DXR were investigated for their cell growth inhibition effect towards in vitro cultured ovarian cancer cells. In the first series of experiments, monolayers of cells were incubated with free DXR or DXR-immunoliposomes. However, only minor cell growth inhibition was observed owing to insufficient cell binding of immunoliposomes (data not shown). To improve the degree of cell binding and to mimic a more therapeutically relevant situation, the immunoliposomes were incubated with the tumour cells in suspension. The cell growth inhibition of DXR-immunoliposomes (IC₅₀ 7.0 ± 4.3 µM) was in the same range as that of free DXR (IC₅₀ 19.4 ± 6.4 µM). The in vitro cell growth inhibition of non-targeted DXR-liposomes (lacking the antibody) was clearly inferior (IC₅₀ 63.0 ± 10.0 µM) to that of the DXR-immunoliposomes (IC₅₀ 19.4 ± 6.4 µM). Empty immunoliposomes did not influence the cell growth at the relevant lipid concentrations used.
In vivo

0.2
0,6

Day 1
Day 3

intact

0.4

0.67 mg DXR kg⁻¹) examined, both types of DXR-liposomes already induced substantial tumour growth inhibition (P< 2 x 10⁻⁵ vs control). A nearly complete inhibition of tumour growth was obtained at dose levels ≥2 mg kg⁻¹. Microscopic differentiation showed that when the determined tumour load was less than 0.5 g, other cells (e.g. lymphocytes) rather than viable tumour cells were responsible for the determined weight.

In vivo distribution of immunoliposomes

To investigate why tumour-specific DXR-immunoliposomes are not more efficacious in vivo than non-targeted DXR-liposomes, the degree of binding of immunoliposomes to the peritoneal tumour cells was determined. These experiments were performed with double-radiolabelled liposomes. [³H]Cholesteryl oleyl ether was used as a marker of the liposomal lipid phase. In addition, [¹⁴C]sucrose was incorporated in the internal aqueous phase to study the integrity of the liposomes. Double-radiolabelled immunoliposomes and non-targeted liposomes were administered i.p. to OVCAR-3-bearing nude mice at different times (day 7, 14 or 21) after tumour cell inoculation. On all days of examination, the cell binding of immunoliposomes was much higher compared with non-targeted liposomes (see Table 1). About 5% of the injected [³H]liposomal dose was found in spleen, liver and blood, 5 h after injection. The integrity of the liposomes with respect to release of the aqueous contents was also investigated. Release of sucrose from the liposomes will be detected as an increase in the [³H]/¹⁴C ratio of the injected liposome preparation. It was calculated that the loss of sucrose label from both immunoliposomes and non-targeted liposomes present in the peritoneal cavity was minimal (<15%) 5 h after injection, indicating that the bilayer structure of the liposomes remained intact (results not shown).

Figure 1 In vitro cell binding of DXR-immunoliposomes. OVCAR-3 cells were incubated with DXR-immunoliposomes (O and ■) or unconjugated DXR-liposomes (□ and □) for 30 (O, □) or 90 min (■, □) at 37°C. After washing the cells to remove unbound liposomes, cell-associated radioactivity was determined as described under Materials and methods. The degree of cell binding is expressed as the estimated amount of liposomes (nmol TL) bound per 10⁶ cells. The Fab/lipid ratio of the immunoliposomes was 12 µg OV-TL3 per µmol TL. The outcome of a representative experiment is shown.

Figure 2 Growth of OVCAR-3 in NMRI nude mice. Mice were injected i.p. with 2 x 10⁷ OVCAR-3 cells (day 0). At days 7, 14 and 21 after tumour cell inoculation, mice were sacrificed for determination of tumour load (ascitic tumour cells □ and solid tumour ○). Values represent the mean tumour load ± s.d. of 8–12 animals.
To investigate the question, whether the fate of the drug, doxorubicin, parallels the fate of the liposomes, the (immuno)liposomes were labelled with \[^{14}C\]DXR and administered i.p. on day 7 after inoculation of the OVCAR-3 cells. Radioactivity associated with the tumour cells was determined 5 and 24 h after injection. As shown in Table II, the percentage cell-bound \[^{14}C\]DXR was not significantly different after administration of non-targeted \[^{14}C\]DXR-liposomes or \[^{14}C\]DXR-immuno-liposomes. Apparently, non-targeted liposomes are as effective as the immunoliposomes in the delivery of DXR to the cancer cells. The extent of DXR associated with the tumour cells after administration of non-targeted \[^{14}C\]DXR-liposomes was much higher than expected on the basis of the levels of cell-associated liposomal \(^3\)H label (see Tables I and II). This observation suggested to us that part of the tumour cell-associated \[^{14}C\]DXR must be attributed to binding of \[^{14}C\]DXR released from the liposomes. To study this possibility, the binding of free DXR to the peritoneal tumour cells was also examined. Free \[^{14}C\]DXR was administered i.p. in ascitic OVCAR-3 tumour-bearing mice on day 21 after tumour cell inoculation. Figure 4 shows that \[^{14}C\]DXR is disappearing from the peritoneal cavity at a slow rate. Even 24 h after injection of \[^{14}C\]DXR 29±4% of the injected dose was recovered from the peritoneal cavity. In line with the earlier suggestion, most of the injected amount of DXR appeared to be associated with the OVCAR-3 cells.

Discussion

The study presented in this paper is concerned with the use of immunoliposomes (antibody-targeted liposomes) as carriers for doxorubicin (DXR) for i.p. chemotherapy of ovarian carcinoma. As a prelude to assessing the anti-tumour activity of DXR-containing OVC-TL3 immunoliposomes towards growing human ovarian cancer cells (OVCAR-3), we first started to evaluate the cytolytic effects and tumour-cell binding of DXR-immunoliposomes towards in vitro cultured OVCAR-3 cells. The in vitro cell growth inhibition of DXR encapsulated in immunoliposomes was far (200-fold) superior to DXR encapsulated in similar non-targeted liposomes (lacking the specific antibody fragments), and almost equalled the efficacy of the free drug. In line with these results, effective tumour cell binding was obtained with OVT-L3 DXR-immunoliposomes and not with non-targeted DXR-liposomes. The cell binding data are in the same Table I

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<th>Tumour load (g)</th>
<th>Injected [^{3}H] dose (%) associated with the tumour cells</th>
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<td></td>
<td>Unconjugated liposomes</td>
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<td>1.0 ± 0.1 (day 7)</td>
<td>17 ± 10</td>
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<tr>
<td>2.7 ± 0.4 (day 14)</td>
<td>6 ± 3</td>
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<td>4.6 ± 0.4 (day 21)</td>
<td>6 ± 1</td>
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(Immunoliposomes containing \[^{3}H\]-cholesteryl oleyl ether were injected i.p. into athymic nude mice, 7, 14 or 21 days after tumour cell inoculation. Radioactivity was determined 5 h after administration as described under Materials and Methods. The Fab'lipid ratio of the immunoliposomes was 30 \(\mu\)g OV-TL3 per \(\mu\)mol TL. Data represent the mean ± S.D. of 4-5 animals. ^*P<0.05; ^**P<0.0001 (immunoliposomes vs unconjugated liposomes on the same day after tumour cell inoculation).
range as those reported earlier obtained in cell binding experiments with OV-TL3 immunoliposomes without DXR (Nüssander et al., 1995), indicating that inclusion of DXR does not interfere with the cell binding of immunoliposomes. As the in vitro binding experiments on tumour cell binding and cytotoxicity of DXR-(immuno)liposomes strongly suggest that specific binding to the cells is conferring greater tumour cytotoxicity, studies were initiated to evaluate the i.p. anti-tumour effects in vivo. The i.p. growing OVCAR-3 tumour was used because of resemblance to human in situ ovarian carcinoma in terms of morphology, histology and synthesis of tumour-specific antigens (Hamilton et al., 1994). The characteristics of ovarian carcinoma, i.e. it usually remains within the peritoneal cavity and rarely disseminates to distant body sites, were confirmed. In an earlier study we reported that OV-TL3 immunoliposomes bind rapidly and efficiently (over 80% of the injected dose on day 21) to tumour cell inoculation in this xenograft model. In vivo target cell binding experiments confirmed these data and showed that the observed degree of tumour cell binding is dependent on the day of administration (i.e. the tumour load). As shown in Figure 3, both DXR-immunoliposomes and non-targeted DXR-liposomes induced a pronounced anti-tumour effect. However, no difference in anti-tumour activity between the targeted and non-targeted formulation was observed. Thus, despite the observation of specific binding of the immunoliposomes to the tumour cells, differences in anti-tumour response exerted by the DXR-immunoliposomes and non-targeted DXR-liposomes were observed only in vitro and not in vivo. One likely explanation for this discrepancy is the occurrence of considerable drug leakage from the administered specific and non-specific DXR-liposomes induced by the peritoneal environment. Rapid, premature drug loss may have overshadowed the occurrence of specific immunoliposome-mediated anti-tumour effects. Studies with radiolabelled DXR showed that practically all DXR recovered from the peritoneal cavity was associated with the tumour cells after i.p. administration of free DXR (Figure 4). Likewise, DXR leaked from DXR-immunoliposomes or non-targeted DXR-liposomes present in the peritoneal cavity will rapidly associate with the tumour cells. The observation that the non-targeted liposomes, which show little cell binding, are able to deliver as much DXR to the target cells as the specific immunoliposomes (Table II) strongly indicates that a large part of the liposomal DXR content is rapidly lost from the non-targeted liposomes. Considering the identical lipid composition, it is reasonable to assume that the extent of DXR leakage was similar for the targeted liposomes. Therefore, leakage and subsequent rapid association with the tumour cells is likely to explain the absence of any differences in anti-tumour response exerted by the immunospecific and non-specific DXR-liposomes and makes it difficult to discriminate between DXR anti-tumour activity induced by cell-bound and non cell-bound liposomes. The fact that differences in tumour cell growth inhibition were observed in vitro and not in vivo probably relates to the different composition of the incubation medium compared with ascites and the much shorter incubation time (30 min) compared with the long residence time in the peritoneal cavity of the mice.

Considering that the majority of the injected DXR molecules associate rapidly with the OVCAR-3 cells after i.p. injection, it is clear that the anti-tumour activity of free DXR is not easily improved by (immuno)liposome-mediated delivery. Other cytostatics with less affinity for the tumour cells should be considered as more promising candidates for immunoliposome-mediated delivery in this tumour model. After cell binding of immunoliposomes, the encapsulated drug can enter the cell via different routes. Firstly, the immunoliposomes can be taken up by endocytosis. However, Nüssander et al. (1995) showed that internalisation by these tumour cells takes place only to a low degree. Secondly, the liposomal contents can be released slowly after tumour cell binding, providing high DXR concentrations near the tumour cell surface. The results presented in this paper indicate that premature drug leakage occurring before binding of the immunoliposomes to the tumour cells plays a more important role in the mechanism of DXR uptake in this tumour model than leakage from cell-bound immunoliposomes. One way to improve the situation for DXR-immunoliposomes in this model is to prevent premature drug leakage by manipulation of the lipid bilayer composition. By the selection of phospholipids with higher phase transition temperatures, more stable DXR-liposomes can be prepared. However, the more rigid DXR-immunoliposomes were not nearly as effective as free DXR and the more fluid-type immunoliposomes used in this study (results not shown). This would indicate to us that cell-bound rigid immunoliposomes do not sufficiently release the encapsulated drug. To improve the therapeutic availability of the entrapped drug, triggered destabilisation of cell-bound rigid immunoliposomes seems a better option. After binding of stable DXR-immunoliposomes to the tumour cells and clearance of unbound DXR-liposomes from the peritoneal cavity, destabilisation by, for example, raising the temperature (then temperature-sensitive liposomes should be used, e.g. Yatvin et al., 1978; Maruyama et al., 1993), or lowering the pH (pH-sensitive liposomes, reviewed by Torchilin et al., 1993) might lead to efficient tumour cell kill.

In summary, the in vitro cell growth inhibition of DXR encapsulated in OV-TL3 immunoliposomes is superior to that of non-targeted DXR-liposomes (lacking the specific antibody). In tumour-bearing nude mice, however, no difference in anti-tumour effect could be determined between targeted and non-targeted DXR-liposomes. Our results indicate that premature DXR leakage from (immuno)liposomes and subsequent DXR association with the tumour cells, explains why no significant differences in anti-tumour activity between DXR-immunoliposomes and non-targeted DXR-liposomes were observed. Further experiments will be focused on the in vitro and in vivo evaluation of stable, temperature-sensitive liposomes, which rapidly release their contents upon raising the temperature to about 42°C.

Abbreviations

CHOL, cholesterol; DMEM, Dulbecco's modified Eagle's medium; DTT, dithiothreitol; DXR, doxorubicin; EPC, egg-phosphatidylcholine; EPG, egg-phosphatidylglycerol; FCS, fetal calf serum; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MPB-PE, N-[4-(p-maleimidophenyl)butyryl] phosphatidylethanolamine; PBS, phosphate-buffered saline; PE, phosphatidylethanolamine; SRB, sulforhodamine-B; TCA, trichloroacetic acid; TL, total lipid (phospholipid + cholesterol).

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References


