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Doxorubicin–PAMAM dendrimer complex attached to liposomes: Cytotoxic studies against human cancer cell lines☆

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Abstract

Liposomes composed of HePC:EPC:SA 10:10:0.1 (molar ratio) (1) and EPC:SA 10:0.1 (molar ratio) (2) were prepared and were used for incorporating the doxorubicin–PAMAM complex (3:1 molar ratio) (3). The doxorubicin–PAMAM complex was attached to liposomes and the incorporation efficiency was 91 and 95% for 1 and 2, respectively. The incorporation efficiency for doxorubicin into PAMAM was almost 97% while doxorubicin to PAMAM molar ratio was 3.56 ± 0.04. The release rate of doxorubicin as doxorubicin–PAMAM complex from liposomes 1 and 2 and from the complex 3, was studied using buffers and 50% RPMI cell culture medium at 37 and 25 °C. The low release rate of doxorubicin as well as the high incorporation efficiency of doxorubicin–PAMAM complex into liposomes are considered as beneficial factors concerning the activity of doxorubicin. The cytotoxic activity of the liposomal formulation 1 incorporating doxorubicin–PAMAM complex, based on doxorubicin activity, was compared to that of 2 incorporating doxorubicin–PAMAM complex and to that of 3. The results showed that complex 1 was the most active formulation against all cancer cell lines compared to that of 2 and 3. Liposomal formulations composed of lipids and of a drug–dendrimer complex could be characterized as modulatory liposomal controlled release system (MLCRS), and could provide benefits to the delivery of drugs and modulate their release.

Keywords: Liposomes; Dendrimers; PAMAM; Doxorubicin; Biological activity

1. Introduction

Liposomes are a non-toxic carrier system mainly for intravenous delivery of drugs and currently are in clinical use. The surface charge and the size of liposomes can modulate their in vivo stability and can

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alter the pharmacokinetic properties of the encapsulated drugs (Allen and Stuart, 1999; Drummond et al., 1999). Hyper-branched polymers are considered as a new class of compounds in comparison to the linear polymers, and have been studied for application in drug delivery. Dendrimers, are considered as highly branched macromolecules; they are small in size, while their low polydispersity can contribute to the reproducibility of their pharmacokinetic behavior (Cloninger, 2002; Aulenta et al., 2003). An ideal dendrimer as drug delivery system must be non-toxic, non-immunogenic and biodegradable (Aulenta et al., 2003). The first complete dendrimer family which has been synthesized, characterized and commercialized is the poly(amidoamine) (PAMAM) dendrimers. They are characterized as safe and non-immunogenic and they are used in drug delivery, delivery of antisense nucleotides and gene therapy, both in vitro and in vivo (Eichman et al., 2001). The bibliography information on the use of dendrimers as drug delivery carriers is limited and only few studies have been published concerning the interaction of dendrimers and drugs (Khuloud et al., 2003). The use of dendrimers as modulators of the release of a drug incorporated into liposomes and the possible alterations of the drug bioavailability seems to be an attractive field for research. Membrane permeable drugs like doxorubicin quickly leak out of the liposomes; however, the development of a modulatory liposomal controlled release system (MLCRS) using a liposomal formulation composed of lipids and a of drug–dendrimer complex, could be a new approach to the field of controlled release systems (Khopade et al., 2002). Hexadecylphosphocholine (HePC) is an antitumor ether lipid (Engelman et al., 1996; Bittman and Arthur, 1999), which is used mainly locally, for the skin metastasis of breast cancer in many European countries. It is accumulated in the membranes of the tumor cells. Its anticancer activity is directly connected to the accumulation of the drug in the cell membrane of the tumor cells. It can form liposomes after the addition of lipidic molecules providing complementary shape to their final structure, which results in lamellar structures (Papagiannaros et al., 2003).

The present work reports on two liposomal formulations which are composed of HePC:Eggphosphatidylcholine:Stearylamine 10:10:0.1 (molar ratio) (1) and Eggphosphatidylcholine:Stearylamine 10:0.1 (molar ratio) (2) incorporating a doxorubicin–PAMAM (3:1 molar ratio) (3) complex. It is of interest that PAMAM dendrimers have been attached to the lipidic bilayers of liposomes composed of hydrogenated soy phospatidylcholine, cholesterol and dicetylphosphate (Khopade et al., 2002) while ‘dendrons’ (partial dendrimers) can be either entrapped in the lipid bilayer, adsorbed on the surface or co-exist between the states mentioned (Purohit et al., 2001). The doxorubicin–PAMAM complex attached to liposome 1 showed a better in vitro cytotoxic activity, compared to that of 2 and 3 as well as a delayed release of doxorubicin that it is essential for reducing its side effects and increasing its therapeutic index. These findings are in accordance to those recently published, that are claimed that the release was lowered for the acidic drug methotrexete (MTX) from a dendrimeric liposomal formulation (Khopade et al., 2002). We designed and synthesized new liposomal formulations 1 and 2 incorporating a doxorubicin–PAMAM complex (3) and evaluated its potential cytotoxicity in vitro, against human cancer cell lines.

2. Material and methods

2.1. Materials

Hexadecylphosphocholine (HePC) was a generous gift from Asta Medica/Baxter (Bielefeld, Germany). Egg yolk phosphatidylcholine (EPC) was purchased from Avanti Polar Lipids (AL, USA). Doxorubicin hydrochloride was purchased from Pharmacia (NJ, USA). PAMAM the poly(amidoamine) fourth generation, TES (*N-tris*(hydroxymethyl)methyl-2-aminoethanesulfonic acid), stearylamine (SA), Sephadex G75, sulphorodamine B (SRB) trichoroacetic acid (TCA) and RPMI 1640 culture medium were purchased from Sigma–Aldrich (Germany). Fetal Calf Serum was obtained from Biochrome (UK). Chloroform, absolute ethanol and methanol were of spectroscopic grade.

2.2. Incorporation and release of doxorubicin from the doxorubicin–PAMAM complex

A given volume (122 μl) of an aqueous solution of doxorubicin was mixed with a PAMAM G4 solution (3:1 and 6:1 molar ratio of doxorubicin–PAMAM)

in methanol (2 ml) and the solutions were stirred for 12 h. The solutions were evaporated to dryness at 30 °C in vacuum and the PAMAM dendrimer incorporating doxorubicin was extracted overnight using chloroform. Chloroform was evaporated to dryness and the dry residue was dissolved in 10 mM acetate buffered solution pH 4.5 or in TES 10 mM pH 7.5 and the absorbance of doxorubicin was measured at $\lambda = 481$ nm using UV–vis. In the later case acidification of the solution and buffering to pH 4.5 was performed before measuring the absorbance. The release of doxorubicin was studied in TES and in acetate buffer at 25 and 37 °C using dialysis bags (molecular weight cut off 13,000).

2.3. Incorporation of doxorubicin–PAMAM complex in liposomes and physicochemical characterization

Doxorubicin–PAMAM complex (3:1 molar ratio) was attached to liposomes, composed either of HePC:EPC:SA 10:10:0.1 (molar ratio) (1) or EPC:SA 10:0.1 (molar ratio) (2); liposomes were prepared by using the thin film hydration method (Gabizon et al., 1998). Briefly, the lipid film was prepared by dissolving HePC (73.6 μmol), EPC (73.6 μmol), SA (0.736 μmol) and doxorubicin–PAMAM complex (2.1 μmol of doxorubicin) and EPC (73.6 μmol) SA (0.736 μmol) and doxorubicin–PAMAM complex (2.1 μmol of doxorubicin) in chloroform. The solvent was slowly evaporated in a flash evaporator to form a lipid film, which was dried under vacuum for at least 12 h. Multilamellar vesicles (MLVs) were prepared by hydrating the lipid film with TES 10 mM pH 7.5 buffer and stirring for 1 h. Small unilamellar vesicles (SUVs) were prepared from the resultant liposomal suspension, which was subjected to sonication for two 5 min periods interrupted by a 5 min resting period, in an ice bath using a probe sonicator (amplitude 100; cycle 0, 7; UP 200S, dr. hielsher GmbH, Berlin, Germany). The resultant vesicles were allowed for 30 min to anneal any structural defects. Non-encapsulated doxorubicin–PAMAM complex was removed by passing the liposomal suspensions through a Sephadex G75 column. The size and ζ-potential of liposomes 1 and 2 incorporating the doxorubicin-PAPAM complex (3:1) (3) were measured using photon correlation spectroscopy (Malvern Zetasizer 3000HS). Doxorubicin concentration was measured on a Perkin-Elmer UV–vis spectrometer at $\lambda = 481$ nm after the addition of absolute ethanol to the samples.

2.4. Release of doxorubicin from the liposomes incorporating doxorubicin–PAMAM complex

The release of doxorubicin from liposomes 1 and 2 incorporating doxorubicin–PAMAM (3:1) complex was studied in buffer TES 10 mM at pH 7.5 and in 50% RPMI culture medium at 25 and 37 °C, by placing the liposomal formulations in dialysis bag (molecular weight cut off 13,000). The doxorubicin released at various times, up to 48 h was measured using UV–vis at $\lambda = 481$ nm.

2.5. In vitro evaluation of the anticancer activity of the liposomes

The cytotoxic activity of all formulations was investigated against various human cancer cell lines (Table 3): DMS114 and NCI-H460 (small cell lung cancer and non small cell lung cancer, respectively), HT29 and HCT116 (colon), MDA-MB435 and MCF7 (breast), SF268 (central nervous system), DU145 (prostate), and SF268 (central nervous system). The cell lines were purchased from the NCI/NIH, USA, and were grown in RPMI-1640 medium supplemented with 10% heat-inactivated Fetal Calf Serum (FCS), 2 mM L-glutamine and antibiotics in a 37 °C humidified incubator and 5% CO_2 atmosphere. Cell viability was assessed by the trypan blue dye exclusion method at the beginning of each experiment and was always greater than 98%. Cells were seeded into 96 well plates in 100 μl/well at densities of 5×10^3 to 20×10^3 cells. Subsequently, the plates were incubated for 24 h to allow adaptation of cells prior to addition of the test agents. At the beginning of each experiment, a plate of each cell line was fixed in situ with TCA and staining with SRB (Monks et al., 1991) to determine a measurement of the cell population for each cell line at the time of drug addition (T_z). Formulations with doxorubicin were added in culture medium, at the same time to each cell line at decreasing concentrations (i.e. four consecutive 10-fold dilutions) ranging from 20 to 0.02 μM of doxorubicin. The cultures were incubated for an additional period of 48 h. The assay was terminated after 48 h by the addition of cold TCA. SRB staining was

performed and absorbance was measured at 530 nm on an EL-311 BIOTEK microelisa reader (Monks et al., 1991). The data represent the mean of three independent experiments performed in triplicates. The parameters GI_{50}, TGI and LC_{50} were determined using our own customized software (Monks et al., 1991). Briefly, the GI_{50} parameter indicates the growth inhibition strength of the test compound and is calculated from the equation: $100 \times (T - T_z)/(C - T_z) = 50$. TGI indicates the cytostatic effect of the compound and is calculated from the equation: $100 \times (T - T_z)/(C - T_z) = 0$. LC_{50} indicates the compound concentration that induces cytotoxicity in 50% of the cells and is calculated from the equation: $100 \times (T - T_0)/T_0 = -50$. T and T_z indicate the absorbance values at the time cells received the tested compound (T_z) and after a period of treatment (T); C indicates the absorbance value measured in untreated cells (Control) after an incubation period equal to the treatment period (Pluquet et al., 2003).

3. Results

3.1. Incorporation and release of doxorubicin from the doxorubicin–PAMAM complex

The formation of the doxorubicin–PAMAM complex (3) has been achieved using two different pH (i.e. TES 10 mM buffer at pH 7.5 or acetate 10 mM acetate at pH 4.5) and different molar ratios of doxorubicin to PAMAM (i.e. 3:1 and 6:1). The results indicate that a doxorubicin to PAMAM molar ratio of 3:1 is sufficient in order to achieve an almost 97% incorporation of doxorubicin into the dendrimer (Table 1). Doxorubicin incorporation into PAMAM is higher when the complex is formatted in TES buffer (pH 7.5) as compared to that of acetate buffer (pH 4.5) (Table 1). The release of doxorubicin appears to be quite slow,

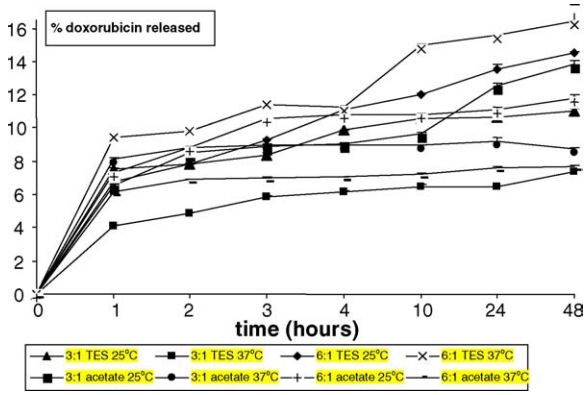

Fig. 1. The release of doxorubicin from doxorubicin–PAMAM complex at a molar ratio of 3:1 and 6:1, in TES and acetate buffers at 25 and 37 °C during a 48 h period. Each point represents the mean of three independent experiments (S.D. never exceeded 5% of the mean value).

7.4% at 37 °C and 11% at 25 °C in TES buffer, and 8.7% at 37 °C and 13.9% at 25 °C in acetate buffer for doxorubicin–PAMAM 3:1 molar ratio (Fig. 1). The release of doxorubicin appears to be also quite slow; 16.5% at 37 °C and 14.6% at 25 °C in TES buffer, and 7.7% at 37 °C and 11.8% at 25 °C in acetate buffer for doxorubicin–PAMAM 6:1 molar ratio (Fig. 1). The lower doxorubicin release (7.4% during 48 h) was observed at a molar ratio of 3:1 of doxorubicin to PAMAM, and the higher (16.5% during 48 h) at molar ratio of 6:1 of doxorubicin to PAMAM in TES buffer (pH 7.5) at 37 °C.

3.2. Incorporation and release of doxorubicin (doxorubicin–PAMAM complex) from liposomes

The incorporation efficiency of doxorubicin–PAMAM complex (molar ratio 3:1) into liposomes 1 and 2 was almost 91 and 95% while doxorubicin

Table 1
Incorporation efficiency (%) of doxorubicin–PAMAM complex at different buffers and different doxorubicin–PAMAM molar ratios

Initial doxorubicin–PAMAM molar ratio (10 mM buffer)	(%) Doxorubicin incorporation		Final doxorubicin–PAMAM molar ratio into the complex	
3:1 (Acetate pH 4.5)	68.91	±0.28	2.54	±0.01
6:1 (Acetate pH 4.5)	35.11	±0.12	2.59	±0.01
3:1 (TES pH 7.5)	96.61	±1.12	3.56	±0.04
6:1 (TES pH 7.5)	50.23	±0.22	3.70	±0.02

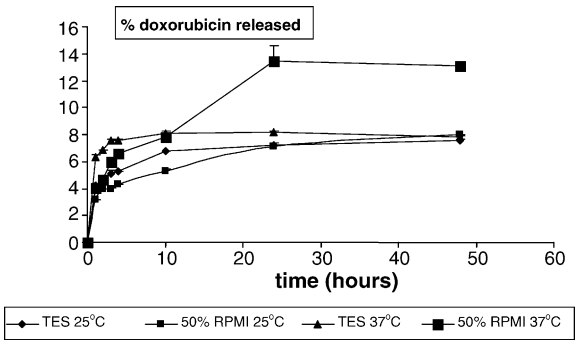

Fig. 2. The release of doxorubicin from doxorubicin–PAMAM complex (3:1 molar ratio), attached to membrane of liposomal formulation 1, in TES and in 50% RPMI culture medium at 25 and 37 °C during a 48 h period. Each point represents the mean of three independent experiments (S.D. never exceeded 5% of the mean value).

[doxorubicin–PAMAM complex (molar ratio 3:1)] to lipid molar ratio was 0.010 (initial 0.014) and 0.020 (initial 0.028), respectively, in TES buffer pH 7.5.

The release of doxorubicin [doxorubicin–PAMAM complex (3:1) (3)] from the liposomes 1 and 2 is quite slow; 7.6% at 25 °C (48 h) and 7.8% at 37 °C (48 h) in TES buffer at pH 7.5, and 8.0% at 25 °C (48 h) and 13.1% at 37 °C (48 h) in 50% RPMI cell culture medium (Fig. 2) for the liposomal formulation 1. For the liposomal formulation 2 the corresponding values were: 10.4% at 25 °C (48 h) and 13.6% at 37 °C (48 h) in TES buffer at pH 7.5, and 17.0% at 25 °C (48 h) and 14.0% at 37 °C (48 h) in 50% RPMI cell culture medium (Fig. 3).

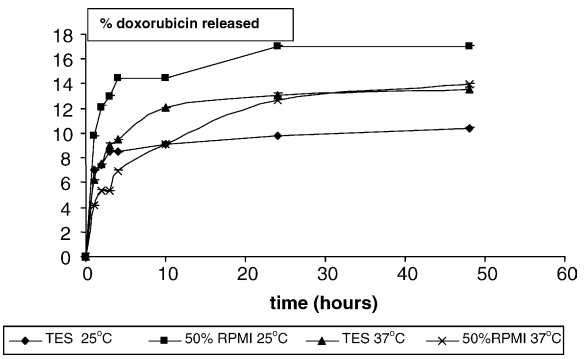

Fig. 3. The release of doxorubicin from the doxorubicin–PAMAM complex (3:1 molar ratio) attached to membrane of liposomal formulation 2 in TES and in 50% RPMI culture medium at 25 and 37 °C during a 48 h period. Each point represents the mean of three independent experiments (S.D. never exceeded 5% of the mean value).

Table 2
Physicochemical characteristics of doxorubicin–PAMAM (3:1 molar ratio) attached to liposomes[a]

Liposome: doxorubicin	PAMAM complex size (nm)	ζ-potential (mV)
HePC:EPC:SA: doxorubicin–PAMAM complex (10:10:0.1): (3:1)	114.9 ± 2.1	-22.0 ± 4.7
EPC:SA: doxorubicin–PAMAM complex (10:0.1): (3:1)	116.3 ± 7.8	-8.7 ± 1.7

[a] Three indepent measurements are run in triplicate.

3.3. Physical stability of liposomes incorporating the doxorubicin–PAMAM complex

Size measurements of the doxorubicin–PAMAM complex (3:1 molar ratio) (3) attached to liposomes 1 and 2, indicate an average size of 114.9 nm and a ζ-potential of -22 ± 4.7 mV, and 116.3 nm and a ζ-potential of -8.7 ± 1.7 mV, respectively (Table 2). The stability of liposomes 1 and 2 was studied for a period up to 26 weeks. The liposomal suspension was kept at 4 °C dark. No sediment was observed while their average hydrodynamic diameter increased rapidly. The average size of liposomes increased up to 500 nm within 3 days and after 4 weeks reached a size of more than 1000 nm while after 18 weeks their average size reached a plateau at 2000 nm in both cases.

3.4. In vitro anticancer activity of the liposomes incorporating doxorubicin–PAMAM complex

All formulations where tested against eight cancerous cell lines originated from different types of cancer. Liposomal formulation 2 was found to be inactive (Table 3) exhibiting only marginal growth inhibiting activity at the highest concentration tested (20 μM). The two colon cancer cell lines HT29 and HCT116 were found to be slightly more sensitive in the treatment with formulation 2 than the rest of the cell lines. The growth inhibiting activity of the doxorubicin–PAMAM complex (3) was found to exhibit a substantially higher activity compared to that of 2 (Table 3), but doxorubicin quickly lost its activity as its concentration in the medium decreased. This formulation found to be more active against one of the two lung cancer cell lines tested the NCI-H460 cells, where at 20 μM it exhibited high cytotoxic activity. Finally, the liposomal formula-

Table 3
Effect of liposomal formulations (1 and 2) and of doxorubicin–PAMAM dendrimer complex (3) against cell lines of different origin

Type of cancer	Cell line	Liposomal formulations								
		1			2			3		
		GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Lung	DMS114	6.2	12.6	18.9	>20	>20	>20	14.0	>20	>20
	NCI-H460	1.3	7.8	>20	>20	>20	>20	11.0	20.0	>20
Colon	HT29	7.0	14.2	21.4	>20	>20	>20	15.8	>20	>20
	HCT116	11.5	20.0	>20	>20	>20	>20	8.8	>20	>20
Breast	MB435	7.3	12.7	18.1	>20	>20	>20	>20	>20	>20
	MCF7	9.6	18.4	>20	>20	>20	>20	12.1	20.0	>20
Prostate	DU145	7.8	16.8	>20	>20	>20	>20	>20	>20	>20
CNS	SF268	15.4	>20	>20	>20	>20	>20	20.0	>20	>20

GI_{50}: Growth inhibiting concentration 50; TGI: total growth inhibition; LC_{50}: lethal concentration 50: concentrations are in μM; S.D. < 15%. Data represent the mean of three independent experiments run in triplicates.

tion 1 was found to be active against the eight cell lines tested, with substantial increase of its cytostatic, cytotoxic and growth inhibiting activity (Table 3). MDA-MB435 breast cancer cells as well as DMS114 and NCI-H460 were found to be the most sensitive in that formulation (Fig. 4) with the latest to be the most sensitive among all cell lines tested. Liposomal formulation 1 decreased the growth rate of these cells even at a concentration of 2 μM as depicted in Fig. 4I.

4. Discussion

Doxorubicin is an anticancer drug usually encapsulated in liposomes by using the pH gradient method (Lasic, 1993). In order to achieve a sufficient controlled release system of doxorubicin from liposomes, a doxorubicin–PAMAM complex (3:1) (3) was prepared and attached to lipidic bilayers of liposomes composed of HePC:EPC:SA 10:10:0.1 (molar ratio) (1) and EPC:SA 10:0.1 (molar ratio) (2) (Khopade et al., 2002).

The results from our study showed that at pH 7.5 (TES buffer) the incorporation efficiency of doxorubicin into PAMAM was greater than that at pH 4.5 (acetate buffer) (i.e. 96.6% versus 68.9%) The reason is that at pH 7.5 (TES buffer) the hydrophobic interactions between PAMAM and doxorubicin are favorable, increasing the incorporation of doxorubicin into PAMAM dendrimer. The tertiary amino groups of PAMAM become protonated below pH 3.7 according to the literature (Kojima et al., 2000). The (%) incorporation efficiency of doxorubicin–PAMAM complex (3:1 molar ratio) (3) into liposomes 1 and 2 was high when the molar ratio of doxorubicin–PAMAM complex was 3:1 either in TES buffer or in acetate buffer, contrary to the low incorporation efficiency when the corresponding molar ratio was 6:1 (Table 1). Consequently, the best amount of doxorubicin is that corresponding to the 3:1 molar ratio of doxorubicin–PAMAM complex. The doxorubicin release rate is an important parameter since a slow release is necessary in order to decrease the unwanted side effects of doxorubicin and improve its therapeutic index (Horovic et al., 1992). Liposomes composed of HePC:EPC:SA 10:10:0.1 (molar ratio) encapsulating doxorubicin exhibited high release rate of doxorubicin (Papagiannaros et al., 2003). On the contrary, doxorubicin incorporated into liposomes 1 and 2 as a doxorubicin–PAMAM complex, exhibited a slow release rate, approximately 14% in the case of a 50% RPMI culture medium at 37 °C after 48 h of incubation period. The effect of temperatures used (i.e. 25 and 37 °C) on the release rate of doxorubicin (doxorubicin–PAMAM complex 3:1 molar ratio) from liposomes 1 and 2, seems not to influence seriously the release of doxorubicin (Figs. 2 and 3). It was from 7.6 to 17% in TES buffer and in 50% RPMI cell culture medium at 25 °C, respectively, and from 7.8 to 14% in TES buffer and in 50% RPMI cell culture medium at 37 °C, respectively. Consequently, the side effects of doxorubicin are expected to be subdued. These observations are in accordance with the release profile of various drugs encapsulated in dendrimers (Kolhe

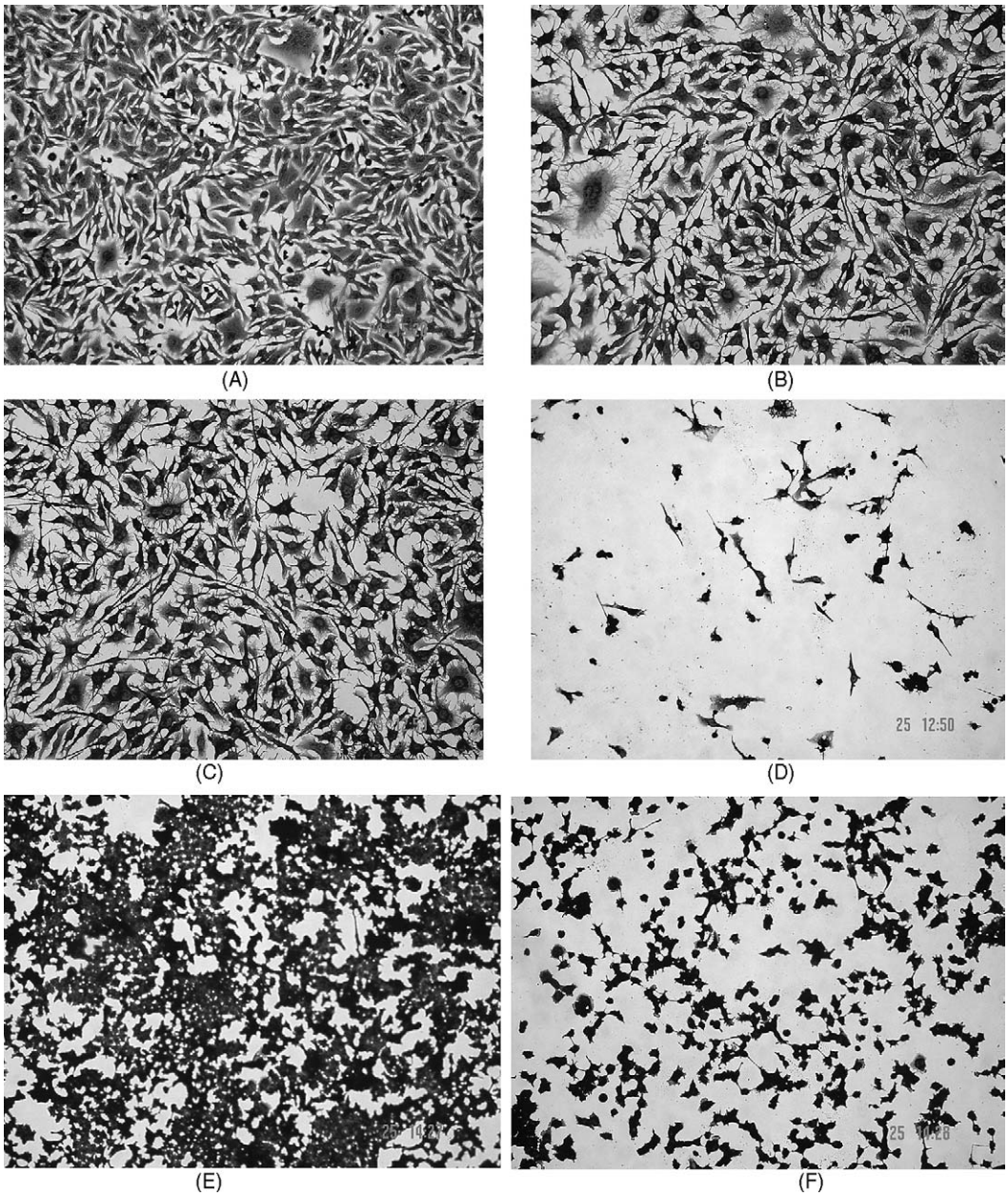

Fig. 4. Morphology of DMS114 and NCI-H460 upon treatment of cells with the three formulations for 48 h. Cells were fixed with TCA and stained with SRB (see text) before being photographed. A–D: DMS114: A: control (untreated); B: treated with 20 μM of 3; C: treated with 20 μM of 2; D: treated with 20 μM of 1. E–I: NCI-H460: E: control (untreated); F: treated with 20 μM of 3; G: treated with 20 μM of 2; H: treated with 20 μM; I: with 2 μM of 1.

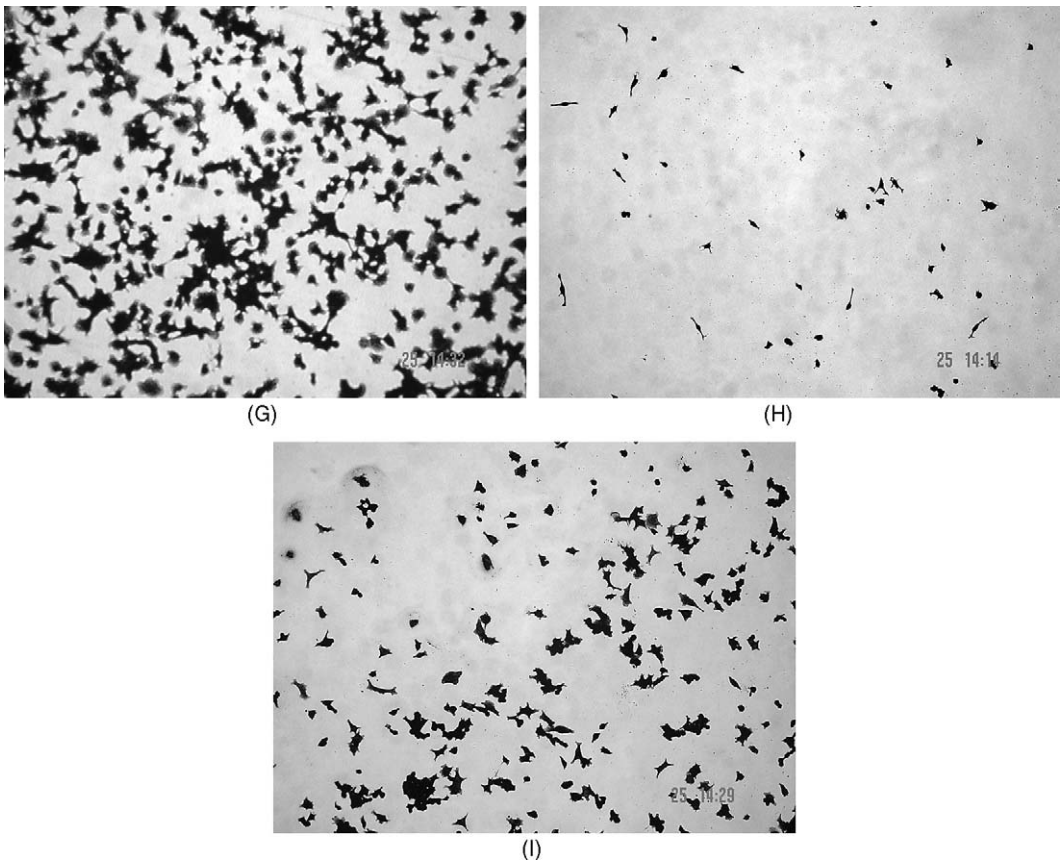

Fig. 4. (*Continued*).

et al., 2003) or incorporated in liposomes together with PAMAM dendrimers (Khopade et al., 2002). The stability of the liposomal formulations 1 and 2, at 4 °C depends on time, as their sizes increased up to 500 nm within 3 days while after 18 weeks their size reached at 2000 nm. The average hydrodynamic diameter of the liposomal formulations 1 and 2, although it was almost 115 nm immediately after the production of the liposomes 1 and 2, increases very rapidly with time. This fact was not observed with the similar composition of liposomes (i.e. HePC:EPC:SA 10:10:0.1, molar ratio) (Papagiannaros et al., 2003), that do not incorporate the doxorubicin–PAMAM complex, and therefore it can be attributed to the presence of the dendrimer. Dendrimer incorporation seems not to affect the liposomal charge of liposomes. It was already found that dendrimers could facilitate the formation of liposome aggregates (Sideratou et al., 2002). The charge of liposomes seems not to play any role to the formation of the aggregates suggesting that hydrophobic forces between dendrimers, which are attached to liposomal particles, may be responsible. Earlier research using 'dendrons' (partial dendrimers) (Purohit et al., 2001) has also reached the same conclusion.

The cytotoxic activity of the liposomal formulations 1 and 2 based on doxorubicin's activity was further tested in vitro against various cell lines. When doxorubicin was incorporated into PAMAM dendrimers retained high growth inhibiting activity which was decreased when the complex attached to EPC:SA liposomes. The activity of doxorubicin was enhanced when HePC was incorporated to the latest formulation. This formulation was superior from all the three formulations against all cell lines tested. It is of interest that the dendrimeric liposomal formulation enhanced the activ-

ity of doxorubicin against MDA-MB435 breast cells. The most sensitive cell lines were those originated from lung cancer, DMS114 and NCI-H460 suggesting a selective action of doxorubicin as compared to the rest of the cell lines. Especially for H460 the growth inhibiting activity of doxorubicin (doxorubicin–PAMAM complex 3:1 molar ratio) attached to liposomal formulation 1 was found to be at about 10 times (one order of magnitude) higher.

5. Conclusion

The pH gradient method has been applied as conventional methodology for encapsulating doxorubicin in liposomes and under this encapsulation methodology the liposomal doxorubicin is already in market. In the present study, we present data regarding the development of two liposomal formulations incorporating a doxorubicin–PAMAM complex. To the best of our knowledge doxorubicin encapsulation into liposomes has never been investigated as a complex of doxorubicin–PAMAM. The liposomal formulations incorporating the doxorubicin–PAMAM complex, exhibited such physicochemical characteristics and release properties, that could be suitable for cytotoxic studies overcoming the fast release of doxorubicin from conventional type of liposomes. The stability of the liposomal formulations based on their size seems to depend on time. The size of liposomes increased rapidly due to the presence of the complex of doxorubicin–PAMAM on the surface of liposomes. The overall procedure seems to be conventional, but the final liposomal formulation seems promising and novel and could be able to increase the therapeutic index of the incorporated drug. The results concerning the cytotoxicity of doxorubicin seems also promising for further cytotoxic studies against cancer cell lines. However, a modulatory liposomal controlled release system (MLCRS) seems to be possible for drug delivery and can modulate the release of drugs from dendrimeric liposomal formulations.

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