DQAsomes: A Novel Potential Drug and Gene Delivery System Made from Dequalinium™

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Purpose. Dequalinium, a drug known for over 30 years, is a dicationic amphiphile compound resembling bolaform electrolytes. The purpose of our work was to determine the state of aggregation of dequalinium in aqueous medium and to investigate both, its ability to bind DNA and its potential to serve as a novel non-viral transfection vector.
Methods. The form of aggregation was determined employing electron microscopic techniques. The DNA binding capacity of dequalinium was assayed using SYBR™ Green I stain. For *in vitro* cell transfection experiments plasmid DNA encoding for firefly luciferase was used.
Results. Dequalinium forms in aqueous medium liposome-like aggregates, which we term DQAsomes. These dequalinium vesicles bind DNA and they are able to transfect cells *in vitro* with an efficiency comparable to Lipofectin™.
Conclusions. Based on the intrinsic properties of dequalinium such as the *in vivo* selectivity for carcinoma cells and selective accumulation in mitochondria we propose DQAsomes as a novel and unique drug and gene delivery system.

KEY WORDS: dequalinium; liposome; bolaform drug; non-viral transfection vector; gene therapy; drug delivery.

INTRODUCTION

Dequalinium (1) has been used for over 30 years as a topical antimicrobial agent. There is no consensus about the molecular target of dequalinium (DQA), although several different targets such as the small conductance Ca^{2+}-activated K^+ channel, F1-ATPase, calmodulin and proteinase K have been suggested (2–5). DQA also shows anticarcinoma activity (6–8). The drug has been shown to accumulate selectively in carcinoma cells versus non-transformed epithelial cells. In addition, the exclusive localization of DQA in mitochondria has been demonstrated (6,7,9,10).

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Here we report that DQA forms liposome-like aggregates upon sonication in water, which we term DQAsomes. We demonstrate both, the ability of DQAsomes to bind DNA and to transfect cells *in vitro* with an efficiency comparable to Lipofectin™.

MATERIALS AND METHODS

Materials

Dequalinium was purchased from Sigma Chemical Co., St. Louis, MO and from Aldrich Chemicals, Milwaukee, WI. SYBR™ Green I was obtained from FMC BioProducts, Rockland, ME, pGL3 firefly luciferase plasmid DNA from Promega, WI.

DQAsome Preparation

26 mg dequalinium chloride were dissolved in a round bottom flask in about 2 ml methanol followed by removing the organic solvent with a rotary evaporator. 5 ml water was added and using a probe sonicator the dequalinium suspension was sonicated until a clear opalescent solution was obtained.

Electron Microscopy

Carbon films were evaporated on freshly cleaved mica and floated free on water. The films were placed on small mesh copper grids which had been previously treated with "grid glue" (one half inch of Scotch tape dissolved in 10 ml chloroform). Suspensions of the sample were allowed to adsorb to the surface of the carbon film. Samples were then treated in one of two ways. Some were negatively stained using 1% aqueous uranyl acetate. Others were allowed to dry and subsequently rotary shadowed with carbon-platinum at an angle of 10 degrees using a Balzers MED 010 vacuum evaporator.

Freeze Fracture Electron Microscopy

The sample was suspended in 10% (v/v) glycerol, placed between copper platelets (sandwich-technique) and plunge-frozen from room temperature using liquefied propane cooled by liquid nitrogen. Fracturing and shadowing was performed at −150°C in a BAF 400T instrument (Balzers, Liechtenstein). Replicas were cleaned with methanol-water (9:1). Electron micrographs were obtained on a CEM 902A instrument (Zeiss, Germany).

Size Distribution Measurements

Vesicles were sized by photon correlation spectroscopy with the Coulter™ Model N4MD sub-micron particle analyzer with size distribution analysis processor and multiple angle detection (Coulter Electronics, Hialeah, Florida, USA).

Binding of Plasmid DNA to DQAsomes

2.7 μg pGL3 firefly luciferase plasmid DNA and the appropriate amount of DQAsomes were incubated for 30 min-

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utes in 500 µl PBS followed by adding 500 µl of a 1 : 10,000 PBS dilution of SYBR Green I stock. After 30 minutes the fluorescence was measured with a PE LS5OB Spectrophotometer at excitation wavelength of 497 nm and emission wavelength of 520 nm.

Transfection Assay

LLPCK1 cells (75% confluence) were transfected by adding 100 µl of a complex of 3 µg firefly luciferase plasmid DNA and vesicles (DQAsomes or DOTAP/DOPE, weight ratios as described in legend) to 1 ml cell suspension in serum free medium. Five hours after adding the transfection mixture, the serum free medium was replaced by serum containing RPMI. Transfected cells were incubated additional 24 hours before the expression of the reporter gene was measured according to the protocol provided by Promega, Madison, WI using a Monolight luminometer from Analytical Luminescence Laboratory, San Diego, CA. For protein determination the BCA assay was used.

RESULTS AND DISCUSSION

Despite the wide use of DQA in research and in pharmaceutical practice, the association properties of this amphiphilic molecule in aqueous medium have rarely been addressed. DQA is a dicationic compound resembling bolaform electrolytes, that is, they are symmetrical molecules with two charge centers separated at a relatively large distance (Fig.1). Attwood concluded from light scattering, surface tension and conductivity measurements that DQA and other bolaform drugs form micelles in aqueous solution (11). The size of these "putative" micelles was not reported. Using electron microscopy (Fig.2) and photon correlation spectroscopy (Fig.3) we found that DQA forms upon sonication spheric appearing aggregates with an diameter between about 70 and

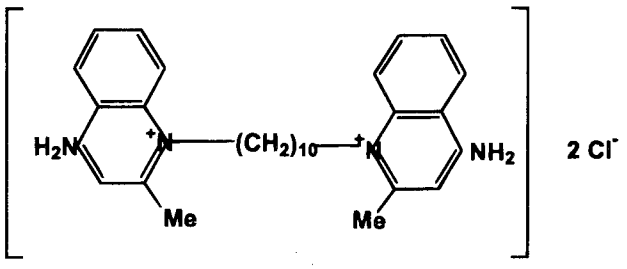

Fig. 1. Chemical structure of dequalinium chloride.

700 nm. This diameter lies well in the broad range known from phospholipid vesicles (12). In contrast, if DQA formed a micelle without an internal aqueous compartment, the diameter would be an order of magnitude lower. Freeze fracture images (Fig.2, panel C) show both, convex and concave fracture faces. These images strongly indicate the liposome-like aggregation of DQA. Negatively stained samples (Fig.2, panel A) demonstrate that the vesicle is impervious to the stain and appears as a clear area surrounded by stain with no substructure visible. Rotary shadowed vesicles (Fig.2, panel B) became very electron dense and showed no substructure. They appear to be dome shaped, but most likely have collapsed during drying.

Particle size measurements of DQAsomes stored at room temperature for 24 and 96 hours (Fig.3) do not show significant changes in their size distribution in comparison to freshly made vesicles measured after one hour. This indicates that DQAsomes do not seem to precipitate, to fuse which each other or to aggregate in solution over a period of several days.

To assess the binding of DNA to DQAsomes we have used the DNA specific dye SYBR™ Green I. The fluorescent signal of this dye is greatly enhanced when bound to DNA, non-binding results in loss of fluorescence. Figure 4 demon-

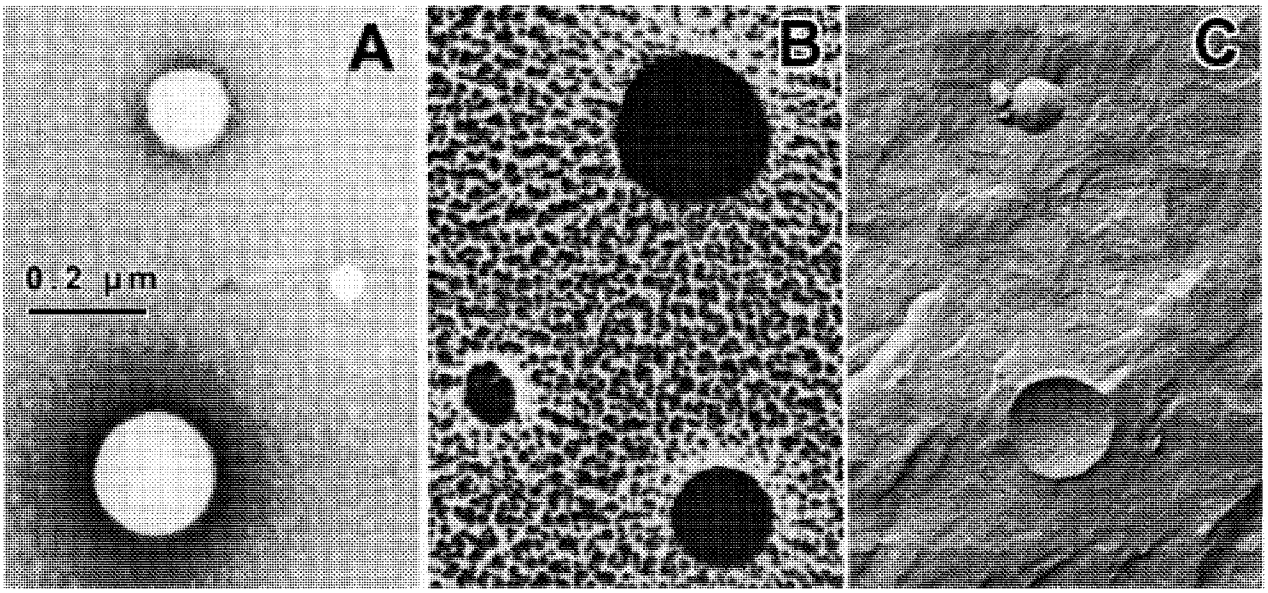

Fig. 2. Electron photomicrograph of DQAsomes. Panel A, negatively stained; Panel B, rotary shadowed; Panel C, freeze fractured.

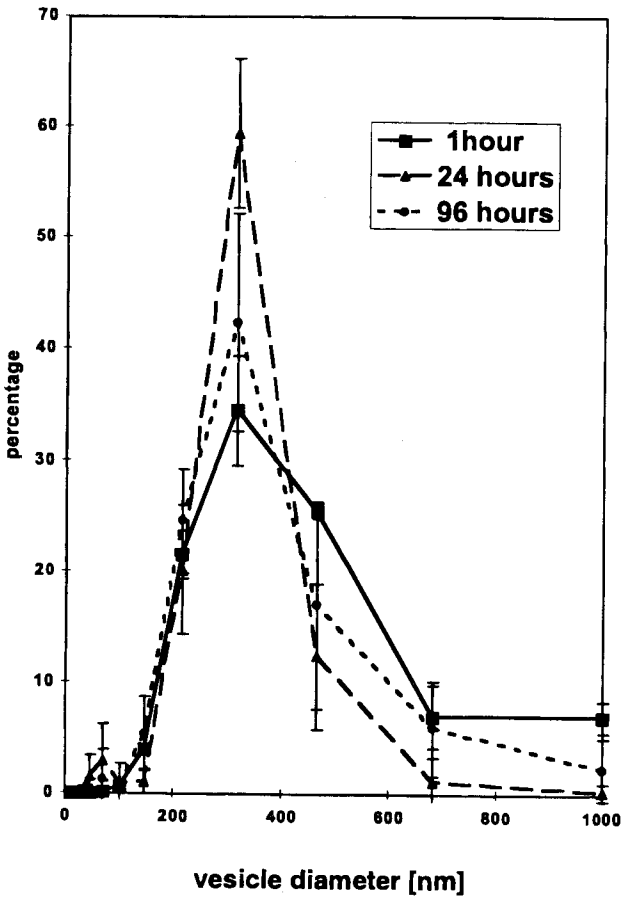

Fig. 3. Size distribution of DQAsomes prepared from dequalinium chloride in distilled water. The size of three independent preparations was determined after storage at room temperature for the number of hours indicated.

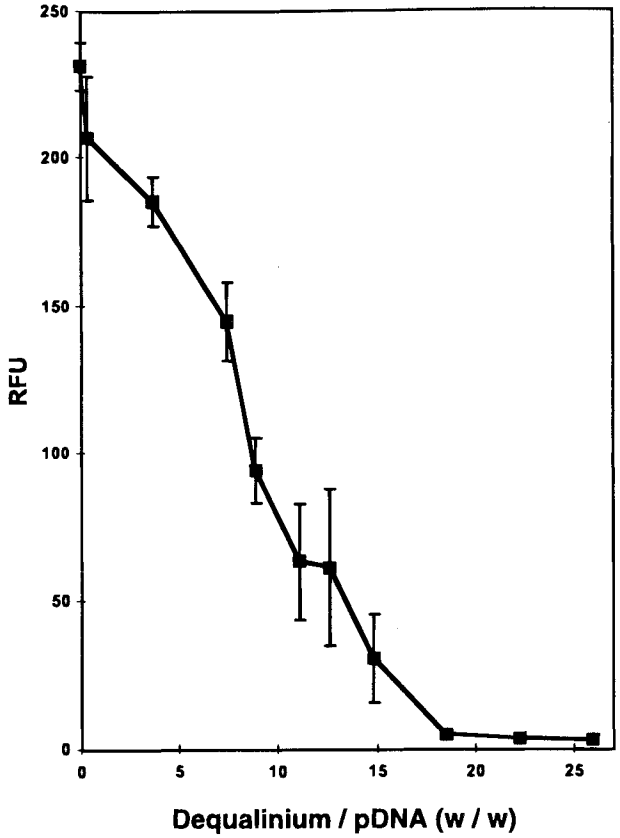

Fig. 4. Binding of plasmid DNA to DQAsomes. DNA was incubated with an increasing amount of DQAsomes followed by adding SYBR™ Green I. Fluorescence expressed as relative fluorescence units (RFU) was measured at excitation wavelength of 497 nm and emission wavelength of 520 nm. For details see MATERIALS AND METHODS.

strates that DQAsomes strongly interact with plasmid DNA. Increasing amounts of DQAsomes prevent SYBR™ from binding to the DNA leading to a complete loss of the fluorescence signal.

As a model for transfection plasmid DNA pGL3 luciferase firefly with SV-40 promotor was used. The expression of the reporter gene became measurable at an approximately mass ratio of dequalinium to DNA of 3.5 (Fig. 5). Maximal expression was achieved at a ratio of 14. Interestingly, at this mass ratio of dequalinium to DNA the plasmid is almost completely prevented from interacting with the fluorescent dye, compare with Figure 4. However, further doubling the amount of DQAsomes lead to a sudden decrease in expression, probably caused by insetting cytotoxicity at this drug concentration. Under our experimental conditions and at the optimal dequalinium to DNA ratio of 14 the transfection efficiency of DQAsomes was comparable to the efficiency of Lipofectin™ (empty bar in Fig. 5).

Based on the intrinsic properties of DQA such as the *in vivo* selectivity for carcinoma cells and selective accumulation in mitochondria we propose DQAsomes as a novel and unique drug and gene delivery system. Studies about mitochondria-specific transfection using DQAsomes as a vector are in progress (Patent pending).

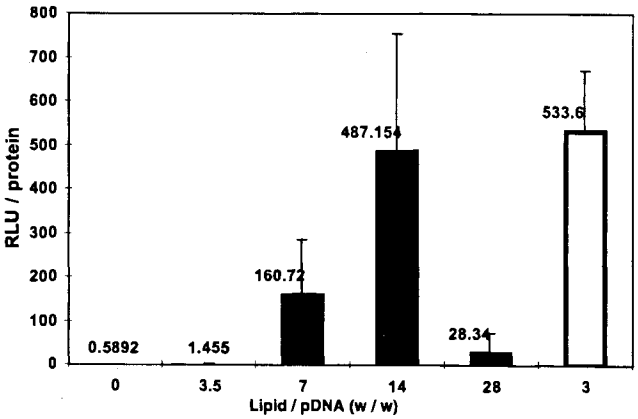

Fig. 5. Transfection efficiency of DQAsomes (filled bars) and DOTAP/DOPE (1:1) (empty bar) at indicated weight ratios of lipid to DNA. For details see MATERIALS AND METHODS.

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