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Original article

Trypanocidal activity of arsonoliposomes: Effect of vesicle lipid composition

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Abstract

Sonicated arsonoliposomes were prepared using an arsonolipid with palmitic acid acyl chain (C16), mixed with phosphatidylcholine (PC-based) or 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC-based), and cholesterol (Chol) with a molar ratio C16 /PC or DSPC/ Chol 8:12:10. PEG-lipid (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine conjugated to polyethylenoglycol 2000) containing vesicles (pegylated-arsonoliposomes) were also prepared. The *in vitro* and *in vivo* trypanocidal activity of the various types of arsonoliposomes was evaluated.

Although PC-based arsonoliposomes exhibited *in vivo* activity on an acute trypanosomiasis animal model, no evidence of activity was demonstrated for DSPC-based or pegylated-arsonoliposomes on a chronic model. Despite the fact that DSPC-based and pegylated-arsonoliposomes have better bioavailability compared to PC-based ones, their *in vitro* activity is lower than that of PC-based arsonoliposomes, indicating the importance of arsonoliposome lipid composition on their trypanocidal activity and suggesting that further arsonoliposome structure design is required to overcome these disadvantages.

Keywords: Liposome; Arsonolipid; Arsenic; *In vivo*; Trypanocidal activity; *Trypanosoma*; Toxicity; Arsonoliposome

1. Introduction

Arsonolipid-containing liposomes called arsonoliposomes (Fig. 1) were prepared and characterized in our laboratory [1]. As recently summarized [2], promising results were obtained with some of the arsonoliposomes prepared, for which a differential toxicity towards cancer and normal cells was demonstrated [3,4] as well as *in vitro* antiparasitic activity [5,6]. Indeed, arsonoliposomes composed of C16-arsonolipid (structure presented in Fig. 1) mixed with phosphatidylcholine (PC) and cholesterol (Chol) [that will be referred to as PC-based arsonoliposomes], were found to posses *in vitro* trypanocidal activity against acute and chronic *Trypanosoma brucei* strains [5]. Since it is well known that parasites from the Trypanosomatidae family such as *T. brucei* cause a variety of important diseases in humans and are responsible for considerable human mortality in developing countries [7], the potential of arsonoliposomes as antiprotozoal therapeutics is furthermore explored in this investigation.

The *in vivo* distribution of arsenic (in mice) after i.p. injection of PC-based arsonoliposomes was also previously studied [8] and the low arsenic distribution observed, especially in blood, was attributed to the physical instability of the specific type of arsonoliposomes used. Recently, it was found that arsonoliposome membrane integrity and size distribution

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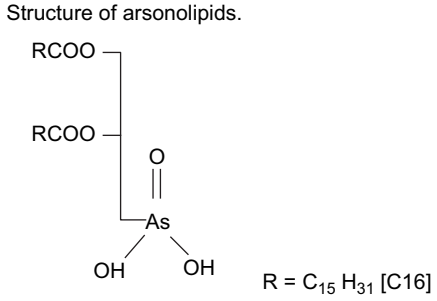

Fig. 1. Structure of arsonolipids.

stability can be influenced by their lipid composition; 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC)-containing arsonoliposomes [DSPC-based arsonoliposomes] were found to have increased integrity in the presence of plasma proteins compared to PC-based ones [9]. Furthermore, when PEG-conjugated lipids were included in the lipid membranes of DSPC-based arsonoliposomes (pegylated-arsonoliposomes) their integrity was further increased [10].

As mentioned above, in a previous *in vitro* investigation [5,6], PC-based arsonoliposomes were found to exhibit trypanocidal activity (MEC 0.2 μM arsonolipid) justifying further *in vivo* exploitation. Therefore, we studied herein, the *in vivo* trypanocidal activity of PC-based arsonoliposomes on an acute infection trypanosomiasis model.

In order to evaluate the effect of the lipid composition of arsonoliposomes on their trypanocidal activity, following the first *in vivo* experiment, the *in vitro* trypanocidal activity of DSPC-based and pegylated-arsonoliposomes was evaluated, using the same experimental setup which was used previously [5], when the activity of PC-based arsonoliposomes was studied, for activity comparison. Since, these latter arsonoliposome types were found to be more stable in the presence of serum [10]; we anticipated that due to enhanced contact time between the parasite and the arsonoliposome, they would be also more efficient in killing bloodstream parasites. This is why, in parallel with the *in vitro* trypanocidal activity DSPC-based and pegylated-arsonoliposomes were also evaluated *in vivo* on a chronic trypanosomiasis model that is a better model for human trypanosomiasis.

2. Materials and methods

2.1. Reagents

Egg L-α-phosphatidylcholine [PC] (grade 1), 1,2-distearoyl-*sn*-glycero-3-phosphocholine [DSPC] (synthetic, grade 1), and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolanine conjugated to polyethylene glycol (MW 2000) [DSPE–PEG_{2000}] (synthetic, grade 1) were purchased from Avanti Polar Lipids. Cholesterol [Chol] (pure) and Triton X-100 were obtained from Sigma–Aldrich (O.M.), Athens, Greece. The water used was deionized and then distilled.

The *rac*-arsonolipid [C16] (2,3-dipalmitoyloxypropylarsonic acid) with a palmitic side chain ($R = C_{15}H_{31}$) was synthesized and characterized, as described in detail previously [11,12].

2.2. Liposome composition

Using the arsonolipid C16 and PC or DSPC, and in some cases DSPE–PEG_{2000}, we prepared liposomes with the following lipid compositions: (a) PC-based arsonoliposomes (PC/Ars/Chol 12:8:10 [mol/mol/mol]), (b) DSPC-based arsonoliposomes (DSPC/Ars/Chol 12:8:10 [mol/mol/mol]), and (c) pegylated-arsonoliposomes (DSPC/Ars/Chol 12:8:10 [mol/mol/mol] with 8 mol% DSPE–PEG_{2000} incorporated).

2.3. Preparation of liposomes

Arsonolipid-containing liposomes were prepared as described previously [1]. In brief, lipids (after removing the organic solvents from the appropriate volume of the lipid solutions, under a nitrogen stream) were mixed with 5 mM phosphate buffer (pH 7.4) and 20 mM NaCl and magnetically stirred vigorously on a hot plate for 4 h at 70–80 °C. After the formation of liposomes, the samples were left to anneal for at least 1 h at the liposome preparation temperature.

In order to reduce liposome size, the large liposome suspension initially produced was sonicated, using a Vibra-cell probe sonicator (Sonics and Materials, UK) equipped with a tapered tip, for at least two 5 min cycles. In all cases the initially turbid liposomal suspension was well clarified after sonication. Following sonication, the liposome suspensions were left to stand for 1 h at 65 °C (or higher than the transition temperature of the lipid used in each case), in order to anneal any structural defects. The titanium fragments and any multilamellar vesicles or liposomal aggregates were removed by centrifugation at 10,000g for 10 min.

2.4. Liposome characterization

The liposomes prepared were characterized by measuring their size by DLS (dynamic light spectroscopy) with a Malvern Zetasizer 5000 (Malvern, UK), as described previously [1]. In brief, liposome dispersions were diluted with filtered phosphate-buffered saline (PBS), pH 7.40 and sized immediately.

The arsonoliposome electrophoretic mobility was also measured at 25 °C (Zetasizer 5000 Malvern Instruments, UK), after diluting the vesicle dispersion with filtered PBS, pH 7.40. ζ-potentials of the dispersions were calculated (by the application of the Helmholtz–Smolowkovski equation).

2.5. Quantification of arsenic in arsonoliposomes

2.5.1. Sample preparation

The arsenic content of arsonoliposomes (by which the arsonolipid content was measured) was determined using atomic absorption spectrophotometry after digestion with fuming nitric acid, as previously reported [13,14]. In brief, 20 μl of the liposome dispersion was digested in 12 ml of nitric acid in a flask. The flask was heated on hot plate placed under