5-Fluoroorotate: a new liposome-dependent cytotoxic agent

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The potency of 5-fluoroorotate for inhibition of L929 or CV1-P cell growth is increased by encapsulation in negatively charged liposomes. The optimal liposome composition is dipalmitoylphosphatidylglycerol: cholesterol, 67:33. Unextruded large unilamellar liposomes are the optimal size for delivery. This compound is the second transport-negative drug which we have found to exhibit liposome-dependent delivery.

Fluoropyrimidine *Liposome* *Targeting* *Endocytosis* *Lysosomotropism* *Drug carrier*

1. INTRODUCTION

We have previously used liposomes to increase the potency of drugs that are transported slowly into cells [1]. 5-Fluoroorotate is a derivative of 5-fluorouracil for which no cellular transport system exists [2]. 5-Fluoroorotate is also a weak acid, and might enter the cytoplasm more readily by escape from the lysosomal compartment after delivery by liposomes [3,4]. Therefore, we have investigated whether the potency of this drug is increased by encapsulation in negatively charged liposomes.

2. MATERIALS AND METHODS

Sodium 5-fluoroorotate (Pharmacia, NJ) gave a maximum concentration of 15 mM, while the lithium salt was soluble to at least 50 mM. For encapsulation, a 50 mM 5-fluoroorotate, pH 7.4, 290 mosmol/kg solution was prepared, containing 50 mM morpholinoethanesulfonate, 50 mM morpholinopropanesulfonate, chloride, and lithium as the counterion. For gel chromatography and subsequent dilution of the liposomes, an equivalent buffer lacking drug was prepared. All solutions were sterilised by filtration prior to use.

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All phospholipids (Avanti, Birmingham, AL) were used without further purification. Cholesterol (Sigma, St Louis) was recrystallised 4 times from methanol. All lipids were stored under argon in chloroform solution in sealed ampoules until use. Liposomes were prepared by reverse-phase evaporation [5], and extrusion [6]. Small liposomes were made by extensive sonication of lipid suspensions. The unencapsulated drug was removed by gel filtration with Sephadex G-75 (Pharmacia). Lipid concentration was measured by phosphorus analysis [7]. The encapsulated drug was measured using a molar extinction coefficient of 7100 in 0.1 N HCl. A liposome sample was extracted [8], the upper phase was acidified with HCl, and its absorbance was measured. L929 [1] and CV1-P [9] cells were obtained and grown as previously described. The IC_{50} of the liposome preparations was measured by growth inhibition as in [1]. The cells were incubated for 48 h (L929) or 72 h (CV1-P) before counting.

3. RESULTS

The captured aqueous volumes are within the expected range for the liposome preparations [6]. This suggests that the drug is encapsulated within the aqueous phase, and does not leak very rapidly

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Table 1

Growth inhibition by 5-fluoroorotate

Lipid[b]	Liposome properties			IC_{50}[a] (μM)	
	Molar ratio	Size[c] (μm)	Capture[d] (l/mol)	CV1-P	L929
Free drug[e]	–	–	–	7 ± 2	1.1 ± 0.3
PG:Chol	67:33	U	9.0	2.0	0.6
		0.1	2.8	2.2	0.6
DSPG:Chol	67:33	U	2.8	0.5	0.72
		SUV	0.8	0.7	0.96
DPPG:Chol	67:33	U	5.2	0.2	0.08
		0.1	3.2	0.2	0.13
		SUV	1.2	1.7	0.8

[a] Concentration of the drug that inhibits cell growth by 50%

[b] Lipids used: Chol, cholesterol; PG, egg phosphatidylglycerol; DPPG, dipalmitoylphosphatidylglycerol; DSPG, distearoylphosphatidylglycerol

[c] Liposomes were prepared by reverse-phase evaporation, and were either unextruded (U), or extruded to 0.1 μm (0.1). Liposomes were also prepared by extensive sonication (SUV)

[d] The theoretical aqueous capture is the drug:lipid ratio (mol/mol) × the inverse of the original drug concentration (0.05 M)

[e] The IC_{50} of the free drug is the mean of 3 determinations for CV1-P cells, and of 4 determinations for L929 cells. All other values are derived from individual growth inhibition curves

from the liposomes. The liposomes may be stored for several weeks at 4°C without any change in their potency, which further confirms the stability of the preparations.

Unencapsulated 5-fluoroorotate has an IC_{50} of 7 μM for CV-1P cells and 1 μM for L929 cells (table 1). When encapsulated in egg phosphatidylglycerol:cholesterol, 67:33 liposomes, the potency of 5-fluoroorotate as increased 2–3-fold. However, drug potency was increased 14–35-fold by encapsulation in dipalmitoylphosphatidylglycerol:cholesterol, 67:33 liposomes. Sonicated liposomes of this composition were 10-fold less effective than larger liposomes for drug delivery. Drug in distearoylphosphatidylglycerol:cholesterol, 67:33 unextruded liposomes was a 2–14-times more potent growth inhibitor than free drug. Sonicated liposomes of this composition are less effective than unextruded large liposomes, but the difference is not as large as that between sonicated liposomes and unextruded liposomes that contain dipalmitoylphosphatidylglycerol.

4. DISCUSSION

The liposome dependence of 5-fluoroorotate should assist considerably the study of targeted drug delivery. 5-Fluoroorotate inhibits the contractility of fibroblasts, and may allow us to develop a specific therapy for intraocular proliferative diseases [10].

5-Fluoroorotate may be considered a liposome-dependent derivative of 5-fluorouracil. Both drugs interfere with ribosomal maturation [11,12], and may also be metabolised to fluorodeoxyuridine monophosphate, an inhibitor of thymidylate synthetase [13]. 5-Fluorouracil has a half-time of transmembrane flux of 30 s [14], while 5-fluoroorotate exhibits very slow transmembrane flux [2]. 5-Fluoroorotate may be more useful for use with liposomes than 5-fluorouracil. Attempts to encapsulate 5-fluorouracil have met with limited success. 5-Fluoroorotate appears to be stably incorporated into liposomes, presumably due to its negative charge.

Experiments on the use of liposomes for 5-fluoroorotate delivery have produced two unexpected observations: (i) Liposomes containing dipalmitoylphosphatidylglycerol are more effective for delivery of 5-fluoroorotate than liposomes containing egg phosphatidylglycerol. (ii) Sonicated liposomes are much less effective than larger liposomes for 5-fluoroorotate delivery, while previous studies have shown sonicated liposomes to be the most effective liposomes for delivery of methotrexate or methotrexate-γ-aspartate [15,16]. These observations may be due to the rate of 5-fluoroorotate leakage from liposomes that are in contact with serum. In future studies, we hope to investigate the leakage of 5-fluoroorotate from liposomes, and its delivery by ligand-directed liposomes.

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