Fusogenic liposomes for the intracellular delivery of phosphocreatine

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Purpose

Develop fusogenic liposomal formulation for cellular delivery of phosphocreatine (PCr) and the resultant generation of adenosine triphosphate (ATP), a primary energy source for many cellular processes.

Motivation

1. ATP drives various cellular processes including: 1) muscle contraction, 2) nerve impulse propagation, 3) chemical synthesis, and 4) maintenance of overall cellular energy homeostasis. Cellular energy production is directly relevant to the augmemntation/optimization of waterfighter performance and the protection of warfighter health. These are clearly defined focus areas in the Navy 30 Year S&T Strategic Plan (Dec. 2022)

2. The delivery to and cellular uptake of PCr for the augmentation of ATP production poses a considerable challenge. This arises for several reasons. First, PCr is negatively-charged and does not readily cross the plasma membrane into the cell. Second, creatine (a precursor required for PCr synthesis) dysfunction of membrane-resident creatine transporters (which exists in a number of physiological conditions) prevents PCr from crossing the plasma membrane.

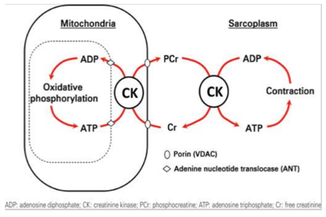
3. Thus, there is a pressing need for technologies that can deliver PCr to the cellular cytosol without the direct interaction of PCr with the plasma membrane.

This invention:

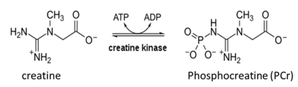
Develops and reduces to practice a series of fusogenic liposomes composed of DOPE, DOTAP, and dye-labeled lipids such as Rhod-PE or NBD-PE. These liposomal preparations were loaded with cell-impermeable chromophores, and PCr; none of these materials are internalized into the cell directly from extracellular medium. PCr delivery resulted in the increased ATP production in live HEK 293T/17 cells (23% in 30 min) incubated with PCr-containing liposomes compared to non-PCr-loaded liposomes.

Prior Art and Limitations

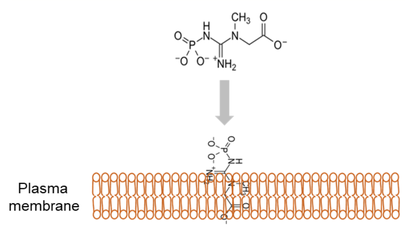
Natural intracellular synthesis of PCr



Einstein (Sao Paulo). 2014 Jan-Mar; 12(1): 126–131.



Extracellular delivery approach of PCr does not work

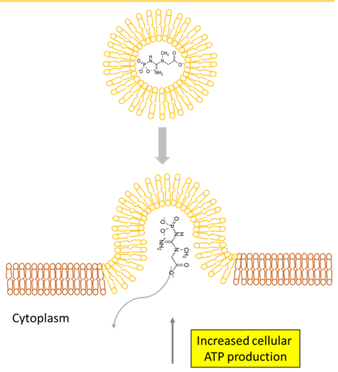


1) PCr is negatively-charged and does not readily cross the plasma membrane into the cell.

2) Creatine (precursor of PCr synthesis) cannot cross the cellular membrane with dysfunction of creatine transporters which exist in a number of physiological conditions

INVENTION

Liposomal membrane fusion medicated delivery of PCr

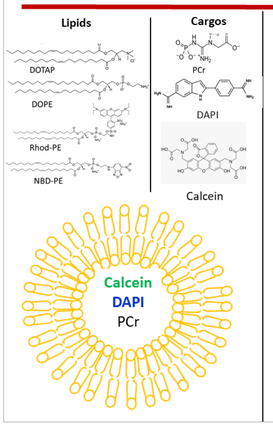


1) This invention details the use of fusogenic liposomes of defined composition as an efficient carrier for delivery of drugs or dyes (*e.g.*, calcein, DAPI) and phosphocreatine (PCr) into living cells.

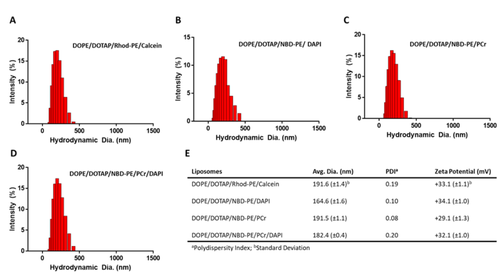
2) Fusogenic liposome-mediated delivery of PCr offers various advantages-
   a) protection of PCr from extracellular hydrolysis
   b) minimize PCr interaction with cellular membrane phospholipids
   c) directly deliver PCr into the cytosol while avoiding other competing internalization pathways, such as endocytosis.

3) The fact that PCr delivered by fusogenic liposomes enters the cytosol and does not significantly interact with or lost to the cellular plasma membrane is a nonobvious feature of the invention given the known nature of PCr interaction with biological membranes.

1) Preparation of fusogenic liposomes and characterizations



Four different fusogenic liposomes with different cargo combinations



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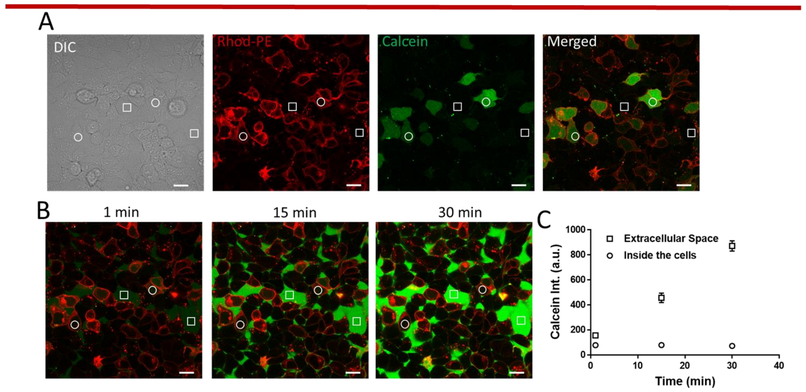
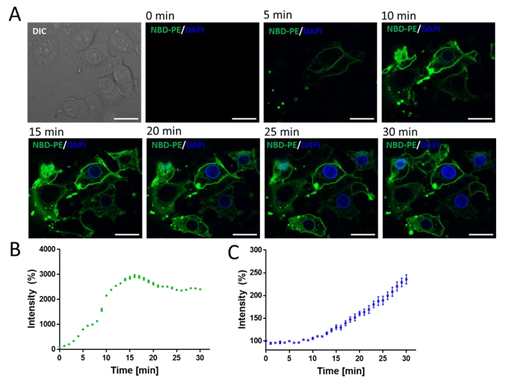
Liposomes	Avg. Dia. (nm)	PDI*	Zeta Potential (mV)
DOPE/DOTAP/Rhod-PE/Calcein	191.6 (±1.4)$^{\$}$	0.19	+33.1 (±1.1)$^{\$}$
DOPE/DOTAP/NBD-PE/DAPI	164.6 (±1.6)	0.10	+34.1 (±1.0)
DOPE/DOTAP/NBD-PE/PCr	191.5 (±1.1)	0.08	+29.1 (±1.3)
DOPE/DOTAP/NBD-PE/PCr/DAPI	182.4 (±0.4)	0.20	+32.1 (±1.0)

*Polydispersity Index; $^{\$}$Standard Deviation

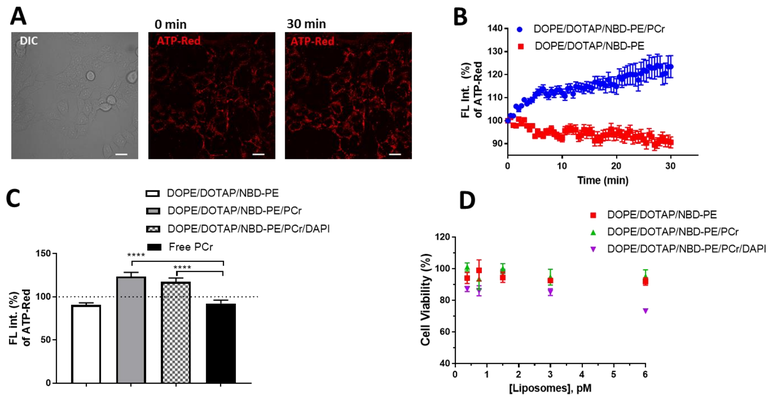
2) Fusogenic liposomes and intracellular delivery of calcein



3) Time-resolved imaging of HEK 293T/17 cells showing clear NBD-PE staining of the plasma membrane confirming successful liposome fusion and DAPI



4) PCr-loaded fusogenic liposomes mediate increased ATP production in HEK 293T/17 cells, and cell viability



Key Advantages and New Features

General:

1) This invention details the use of fusogenic liposomes of defined composition as an efficient carrier for delivery of drugs or dyes (*e.g.*, calcein, DAPI) and phosphocreatine into living cells.

2) Intracellular delivery of PCr offer various advantages, including 1) the protection of PCr from extracellular hydrolysis, the minimization of PCr interaction with cellular membrane phospholipids, and 3) the direct delivery of PCr into the cytosol while avoiding other complication and competing internalization pathways, such as endocytosis that occur when using other types of delivery vehicles (*e.g.*, non-fusogenic liposomes).

3)The fact that PCr delivered by fusogenic liposomes enters the cytosol and does not significantly interact with or is lost to the cellular plasma membrane is a nonobvious feature of the invention given the known tendency for PCr to interact with and be sequestered by the plasma membrane.

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