Cytoprotection by Stabilization of Cell Membranes

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The major constraint that limits tissue storage is the accumulation of cellular damage or wounding by the storage conditions and consequential loss of tissue viability. The noun wound is used in the medical field to refer to a disruption of tissue integrity that follows trauma. Subsequent wound healing results from exposure to supraphysiological forces or is the consequence of action by reactive chemical agents.[1,2] Cell wounding results from exposure to supraphysiological forces or is the consequence of action by reactive chemical agents. Cell wounds result from alteration of cellular moleculary structure or disruption of molecular assemblies such as membranes. Unlike tissue healing, the healing of cellular wounds occurs by neighboring biomolecular interactions. Like tissue wound healing, cellular wound healing involves accelerated processes that are constitutively expressed in routine physiologic repair of cellular structures.[3] This discussion relates to pharmaceutical strategies that are useful for augmenting the cellular healing response.

CELLULAR WOUNDS

Damage to supramolecular assemblies, like the lipid bilayer, is perhaps the most common mode of cell injury during storage.[2] Loss of lipid bilayer integrity occurs after exposure to supraphysiologic temperatures,[4] during freezing and thawing injuries,[5] in free-radical–mediated radiation injury,[6] in barometric trauma,[2,7] and in electrical shock[1,8,9] and mechanical shear or crush forces.[3,10] Ischemia-reperfusion injury, which is mediated by the effects of reactive oxygen species (ROS), is probably the most common cause and is a substantial factor in many common medical illnesses.[6]

Although in each instance the final result is a cell wound, the modes of cellular membrane injury are through different pathways. ROS produces wounding of the cell membrane through peroxidation of phospholipids and oxidative deamination of

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proteins. This altered lipid conformation results in bleb formation, followed by formation of membrane defects. Membrane electroporation results from the pull of water into the membrane by the supraphysiologic electric fields. Heating increases the kinetic energy of membrane amphiphilic lipids until their momentum overcomes the forces of hydration that act to hold the lipids within the membrane lamella. Under freeze conditions, ice nucleation in the cytoplasm can lead to factors that are very destructive to the cell membrane, including the mechanical disruption of the membrane by the ice crystal growth and the damaging effects of increasing salt concentration as the ice spreads and excludes ions.[11] Abrupt barometric pressures can lead to acoustic wave disruption of the cell membrane.[7]

MOLECULAR STRUCTURE–FUNCTION RELATIONSHIP

Molecular structure is critical for the formation of large supramolecular assemblies like the lipid bilayer membrane.[12] Structural integrity of cell membrane is essential for making possible the transmembrane physiological ionic concentration gradients at a metabolic energy cost that is affordable. Despite the effectiveness of the membrane barrier, approximately 40–85% of the metabolic energy expended by cells is used to maintain normal transmembrane ion gradients. Cellular wounds involving disruption of the membrane structure quickly lead to cell necrosis.

DRUGS TO AUGMENT CELL WOUND HEALING

The possibility of sealing cell membrane wounds using synthetic surfactants is now well established.[1,13,14] However, the exact mechanisms of sealing remains unknown.[15] Effective agents include the surfactant class of poloxamers, representing a group of tri-block copolymers. Poloxamer 188 (P188) made by BASF Corporation (Pluronic F68) was initially shown to seal cells against loss of carboxyfluorescein dye after electroporation.[1] In the following years, it has been demonstrated that P188 can also seal membrane pores in skeletal muscle cells after heat shock[16] and enhance the functional recovery of fibroblasts that have been lethally heat-shocked[13] or exposed to high-dose ionizing radiation.[17] Very recently, P188 has been shown to protect embryonic hippocampal neurons against death due to neurotoxin-induced loss of membrane integrity.[14] Other surfactants, such as Poloxamine 1107, of similar composition but quad-block copolymers, have been shown to reduce testicular ischemia-reperfusion injury[6] and hemoglobin leakage from erythrocytes after ionizing radiation.[18] In all the aforementioned investigations the observed phenomena were attributed to sealing of permeabilized cell membranes by the surfactants. In addition, the effect of P188 infusions in reducing duration and severity of acute painful episodes of sickle cell disease is presently also explained by beneficial surfactant–erythrocyte membrane interactions.[19]

Poloxamers and poloxamines belong to a class of water-soluble multi-block copolymers that have important “surface-active” properties. Poloxamer 188 is a triblock copolymer often abbreviated as POE-POP-POE with POE and POP representing poly(oxyethylene) and poly(oxypropylene), respectively. The POE chains are

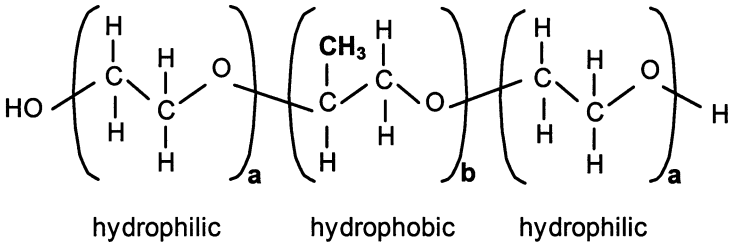

FIGURE 1. Chemical structure of poloxamers. The series of different poloxamers is constituted through varying numbers and ratios for **a** and **b**.

hydrophilic due to their short carbon unit between the oxygen bridges, whereas the POP center is hydrophobic due to the larger propylene unit (FIG. 1).

The poloxamer series covers a range of liquids, pastes, and solids, with molecular weights varying from 1100 to about 14,000. The ethylene oxide propylene oxide weight ratios range from about 1:9 to about 8:2. Poloxamer 188 (P188) has an average molecular weight of about 8400. It is prepared from a 1750 average molecular weight hydrophobe and its hydrophile comprises about 80% of the total molecular weight. The poloxamine series is slightly different from the poloxamer series in its chemical structure. The hydrophobic center consists of two tertiary amino groups carrying both two hydrophobic poly(oxypropylene) chains of equal length each followed by a hydrophilic poly(oxyethylene) chain. Thus, it still can be described as a tri-block copolymer, but is much bulkier than poloxamers (FIG. 2).

Most often, P188 is used at a sub-critical micelle concentration (sub-CMC) of 0.1 mM to1.0 mM for membrane repair *in vitro.*[1] Above their CMC surfactants self-aggregate to micelles causing the (active) surfactant monomer concentration to remain constant (= CMC) independently of the total surfactant concentration . The capability of these amphiphilic copolymers to repair cell membranes at 10^{-3} molar concentration levels distinguishes the sealing capability of copolymer surfactants from purely hydrophilic polymers such as poly(-ethyleneglycol) (PEG), which require molar concentrations.[10]

PEG has a long history of use for induction of membrane fusion applications.[20] It is hypothesized that PEG can force very close contact between vesicle membranes by lowering the activity of water adjacent to the membrane.[12] But even at the required high concentrations (e.g., 17.5%),[20] PEG-mediated vesicle fusion only occurs when the organization of lipid in the bilayers are substantially perturbed from their equilibrium values.

It is our working hypothesis that tri-block copolymers, like P188, with their hydrophobic center chains, act like cell wound targeted PEG molecules, thus requiring much lower concentrations to achieve fusion (sealing) of a permeabilized cell membrane. The membrane repair mechanism of these surfactants is becoming widely discussed. It is not yet understood whether the surfactants interact only with the disrupted parts of the membrane to seal the membrane wounds or whether their integration and interaction with the entire bilayer alters the membrane properties in a way to repair itself (e.g., decreased fluidity).[21,22]

FIGURE 2. Schematic drawing to illustrate structural differences between poloxamers (*left*) and poloxamines (*right*). The PEO and PPO chain lengths vary among the members of the surfactant families.

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