Trends in Transplantation

Commentary ISSN: 1887-455X

Potential Role for Glycerylphosphorylcholine in Cryopreservation

Jack V Greiner[1,2*] and Thomas Glonek[2]

[1]Department of Ophthalmology, Schepens Eye Research Institute of Massachusetts Eye & Ear, Harvard Medical School, Boston, MA, USA
[2]Clinical Eye Research of Boston, Boston, MA, USA

The availability of tissue for transplantation requires preservation adequate to maintain healthy metabolism and cytoarchitecture. Adequate maintenance of the metabolic and cytoarchitectural status of a tissue can be prolonged using cryopreservation techniques. With cryopreservation, the greater the reduction in temperature, the slower the metabolic decline, and the longer tissues can be preserved. However, if the reduction of temperature beyond the freezing-point results in ice formation, it can irreparably damage tissue metabolism and cytoarchitecture. In a preliminary study, it was observed that the freezing point was depressed when cornea tissues were refrigerated at subzero degree temperatures while submerged in preservation medium containing 30 mM glycerylphosphorylcholine (GPC) [1].

GPC is an organophosphate detected by phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P NMR) in corneas of numerous species including humans [2]. Phosphorus-31 is the naturally occurring isotope of phosphorus in the body. In the ^{31}P NMR spectrum of living tissues, the spectral chemical-shift parameter of GPC is essentially unaffected by variations in physiologic pH, ionic strength, or the nature of counter-cations [3]. As such, GPC has been used as a reference marker to calibrate ^{31}P NMR chemical-shift positions and to identify the numerous organophosphates detected in the living tissue ^{31}P NMR spectrum [4]. Ordinarily GPC comprises only a minimal (<5%) amount of the entire organophosphate complement of the ^{31}P NMR spectrum when a tissue is normal and healthy.

Unexpectedly, we discovered an exceedingly high concentration of GPC occurring in the cornea ex vivo, when corneas were incubated at 4°C overnight in a preservation medium supplemented with 30 mM GPC intended as a ^{31}P NMR reference marker [5]. When examined by NMR spectroscopy after 8 hours incubation, the concentration of intracorneal GPC was found to be an order-of-magnitude greater [6] when compared to the levels found in normal fresh corneal tissues [7]. ^{31}P NMR spectroscopic examination of the preservation medium alone revealed an almost undetectable amount of remaining exogenous GPC, suggesting the GPC was absorbed into the cornea. Corneas pretreated in incubation medium containing 30 mM GPC while cooling to subzero degree temperatures were observed to maintain transparency and histological integrity compared to tissues where exogenous GPC was not available [5]. Similar findings were observed when previously GPC-incubated corneas were frozen at liquid nitrogen temperatures [5]. Moreover, it was discovered that accompanying the increase in intracorneal GPC, the metabolic integrity of the cornea as measured by the energy modulus [8,9] was maintained after cryogenic treatment [6].

An analogous increase in the concentration of GPC as measured by ^{31}P NMR was found in ex vivo gastrocnemius muscles obtained from wintering frogs and toads in contrast to those same muscles obtained from the summer-phase animals [10]. Like the corneas, in the muscle tissues, the concentration of GPC was found to be in the range of an order-of-magnitude greater in the (cold) wintering animals relative to their summer-phase counterparts. In one instance, GPC achieved a tissue concentration of 30 mM endogenous GPC. At decreased temperatures there is a decline in metabolic activity. Such a decline permits lowered metabolism and, thus, the potential for cryopreservation. Our recent reports [5,6] provide evidence for such preservation capability. In this work we were able to demonstrate maintenance of function by sustaining tissue transparency and less disruption of histologic integrity at both the light and transmission electron microscopic levels [5].

A cell that has already been shown to possess a high survival rate when preserved long-term at liquid-nitrogen temperatures is sperm. Coincidently, sperm possesses a very high endogenous concentration of GPC under normal circumstances [11,12]. The magnitude of the endogenous GPC concentration in sperm is high and resembles the elevations found in toad and frog muscle during winter [11] and cornea incubated in cold medium containing 30 mM GPC [5,6]. Considering these examples, hypothermia is the common factor. However, unlike the high endogenous levels of GPC in sperm, the cornea and muscle tissues do not usually contain high concentrations of endogenous GPC at ambient temperatures, but appear to be able to concentrate GPC when subjected to low temperatures. At present the concentration mechanism is unknown. These observations support the notion that GPC in high concentrations could act as a cryoprotectant.

Does subjecting ex vivo tissues to exogenous GPC under hypothermic conditions, in the case of the cornea, or in vivo tissues to hypothermic conditions, in the case of toad or frog muscle, stimulate the tissue to absorb and concentrate GPC? Regarding our findings in cornea tissue [5,6], it is important to know what activates or permits absorption of exogenous GPC. Alternatively, in muscles of animals subjected to cold temperature, the tissue itself activates the accumulation of GPC.

When considering GPC as a potential cryoprotectant, the two major barriers to current cryoprotectants are tissue: (1) permeability,

Correspondence to: Greiner, Schepens Eye Research Institute, Massachusetts Eye &Ear, 20 Staniford St. W239, Boston, MA 02114, Tel: 617.742.3140, Ext: 413; Mobile: 781.248.3882; E-mail: Jack_Greiner@meei.Harvard.Edu

Key words: *cornea, cryopreservation, glycerylphosphorylcholine, GPC*

Received: July 14, 2020; **Accepted:** July 23, 2020; **Published:** July 27, 2020

and (2) toxicity at the concentrations required for successful cryoprotectants. Our recent studies [5,6] demonstrated that corneas concentrating large amounts of GPC maintain both transparency and metabolic viability at low temperatures, which supports the concept that endogenous GPC in high concentrations is not toxic. As such, GPC has a demonstrable potential as a cryoprotectant or antifreeze for corneal storage. The fact that the GPC is endogenous to many tissues further supports the premise that it has a low level of toxicity. Even at elevated levels of intracorneal GPC after incubation in preservation medium supplemented with 30 mM GPC, the complement of high-energy phosphatic metabolite levels is maintained in the ex vivo ^{31}P NMR spectrum. This is confirmed by the maintenance of the ^{31}P energy modulus [5].

The permeability of the cornea to GPC, its apparent lack of toxicity [6] and low level of histological disruption [5], along with its apparent ability to hinder formation of ice at temperatures below freezing [1], represents a more optimal avenue for corneal preservation and storage of donor tissues for long duration at very low temperatures. Considering the above, the GPC enhanced preservation medium and absorption of exogenous GPC into the cornea may extend cryopreservation storage of corneas and potentially other body tissues, thereby increasing the inventory of viable tissues for transplantation.

Acknowledgement

Supported in part by the Valarie and Walter Winchester Grant, Schepens Eye Research Institute, Massachusetts Eye & Ear, Harvard Medical School, Boston, MA.

Reference

1. Glonek T, Greiner JV, Korb DR, Olson MC (2002) Retention of corneal transparency and histology following prolonged cryopreservation at subzero degree temperatures. American Academy of Ophthalmology, Annual Meeting, Orlando.

2. Greiner JV, Lass JH, Glonek T (1989) Interspecies analysis of corneal phosphate metabolites. *Exp Eye Res* 49: 523-529.

3. Glonek T, Burt CT, Bárány M (1981) NMR analysis of intact tissues including several examples of normal and diseased human muscle determinations. In: Diehl P, Fluck E, Kosfeld R (Eds) NMR, Basic Principles and Progress, NMR in Medicine, Springer Verlag, Berlin, Heidelberg 19: 120-159.

4. Glonek T, Kopp SJ (1985) Ex vivo P-31 NMR of lens, cornea, heart, and brain. *Magn Reson Imag* 3: 359-376. [Crossref]

5. Greiner JV, Glonek T, Korb DR, Lindsay BA, Oliver PJ, et al. (2020) Corneal cryopreservation using glycerylphosphorylcholine-enriched medium. *Cornea* 39: 370-375. [Crossref]

6. Greiner JV, Glonek T, Korb DR, Lindsay BA, Oliver PJ (2020) Corneal absorption of glycerylphosphorylcholine. *Exp Eye Res* 192: 1079323. [Crossref]

7. Greiner JV, Kopp SJ, Gillette TE, Glonek T (1983) Phosphatic metabolites of the intact cornea by phosphorus-31 nuclear magnetic resonance. *Invest Ophthalmol Vis Sci* 24: 535-542. [Crossref]

8. Greiner JV, Lass JH, Glonek T (1984) Ex vivo metabolic analysis of eye bank corneas using phosphorus nuclear magnetic resonance. *Arch Ophthalmol* 102: 1171-1173. [Crossref]

9. Greiner JV, Kopp SJ, Glonek T (1985) Phosphorus nuclear magnetic resonance and ocular metabolism. *Surv Ophthalmol* 30: 189-202.

10. Burk CT, Glonek T, Bárány, M (1976) Phosphorus-31 nuclear magnetic resonance detection of unexpected phosphodiesters in muscle. *Biochemistry* 15: 4850-4853.

11. Burt CT, Chalovich J (1978) Serine ethanolamine phosphodiester: a major component in chicken semen. *Biochim Biophys Acta* 529: 186-188. [Crossref]

12. Arrata WSM, Burt T, Corder S (1978) The role of phosphate esters in male fertility. *Fertil Steril* 30: 329-333.