Original Research

Effects of ATP administration on isolated swine hearts: Implications for *ex vivo* perfusion and cardiac transplantation

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Impact statement

We employed an isolated swine heart four-chamber working model to investigate two potential strategies for adenosine triphosphate (ATP) administration as an *ex vivo* therapy: (1) application of a single bolus dose during reperfusion (postconditioning or PoC), and (2) repeated bolus dosing throughout the experiment (supplementary or Sup). *Ex vivo* swine hearts in the Sup group elicited significantly higher left ventricular function during the 2 h experimental monitoring period. In contrast, ATP administration in the PoC group appeared to induce a degree of depressed hemodynamic function. These data suggest varied functional roles of ATP administration relative to their use in *ex vivo* perfusion strategies. We consider that both treatment strategies, if appropriately administered and with further investigation of dosing paradigms, may eventually elicit value in various clinical scenarios, including heart transplantation and *ex vivo* heart perfusion to assess potential organs for transplantation and potentially increase the pool of viable donor hearts.

Abstract

Cardiac transplant outcomes can be compromised by the effects of global ischemia and associated reperfusion injury. In attempts to alleviate these phenomena, various pharmaceutical agents can be administered. Previous reports have shown that adenosine triphosphate (ATP) may act as either a postconditioning (PoC) or supplementary (Sup) therapy with cardiosupportive benefits. To further evaluate ATP's relative effectiveness, we used an isolated swine heart four-chamber working model to monitor both hemodynamic and metabolic responses. We employed two strategies of ATP administration: (1) a postconditional (PoC) bolus just prior to reanimation, and (2) regular dosing throughout the assessment period (Sup). *Ex vivo* swine hearts in the Sup group elicited significantly higher left ventricular function during the 2 h monitoring period than controls. In contrast, PoC administration appeared to induce depressed cardiac function. The effects of ATP on cardiac function can have varied effects, dependent on when it is administered.

Keywords: Pharmacologic cardioprotection, adenosine triphosphate, *ex vivo* perfusion, transplantation, postconditioning, ATP supplementation

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Introduction

In 1973, Hearse *et al*. reported that relative to organ transplantation, it is not the duration between explant and implant that causes the greatest decrease in tissue viability, but rather the effects of reperfusion with oxygen.[1] Commonly called the oxygen paradox, this phenomenon has been primarily characterized by increased myoplasmic calcium oscillation in hypoxic cells, leading

to hypercontracture, edema, and apoptosis in neighboring cell populations.[1–3]

In 1986, Murry *et al*. reported that 5 min occlusion of the circumflex artery resulted in resistance to the effects of reperfusion injury.[4] Further study of this phenomenon, known as *ischemic preconditioning* (IPC), revealed that it also decreased arrhythmogenesis and reduced infarct size.[4] Yet, the proposed underlying mechanisms for IPC are considered diverse, including: (1) cell-surface activation; (2) signaling pathways; and/or (3) inhibition of mitochondrial end effectors. These end effector pathways currently serve as a promising substrate for development of various pharmacologic agents to mimic similar mechanisms without the need for occlusion.[5]

In various cardiac ischemic injury experimental models, both IPC and pharmacologic preconditioning paradigms have resulted in delayed or reduced cell death. To date, however, their clinical applicability has been limited to situations in which the ischemic events can be anticipated, such as: (1) on- or off-bypass cardiac surgery; (2) percutaneous transluminal coronary angioplasty; and (3) occlusion during stenting procedures. Of importance, in 2004, these limitations were initially addressed by Hausenloy *et al*. who optimized IPC; these researchers identified that the vulnerable time frame for conditioning could be pushed beyond the time of initial reperfusion, in other words postconditioning (PoC) administration.[6,7]

Our current understanding implicates, in part, the mitochondrial permeability transition pore (mPTP) as a primary end effector; note that its inhibition is the last step in the signal transduction for ischemic protection (Figure 1).[5,8]

Adenosine triphosphate (ATP) is a highly polar and negatively charged purine nucleotide (molecular weight: 507.18 g/mol). Burnstock reported that ATP administration can act as an extracellular signaling modulator by binding to purine receptors,[9] which have also been described as mediators for inflammatory processes[10] and the induction of pharmacologic preconditioning.[2] Relative to phospholipase C, ATP has also been reported to activate $P2Y_1$ receptors; this modulatory action could potentially mediate positive conditioning via the subsequent sensitization of the adenosine receptor A_{2b}.[11] Available adenosine may then initiate the reperfusion injury salvage kinase (RISK) signaling cascade; this ultimately closes the mPTP which is considered a beneficial step of PoC in skeletal muscle.[12] However, little work has been done to define ATP as either a preconditioning or PoC agent relative to its effect on large mammalian hearts, specifically in swine models.

Furthermore, ATP has been reported to potentially function as both an inotropic agent and immunologic moderator, possibly helping to limit vascular injury targeting the $P2X_4$ purinoceptor.[13,14] It should be noted that ATP is rapidly degraded to adenosine in the vasculature by the ecto-ATPases CD39 and CD73; adenosine in turn inhibits the production of cytokines implicated in inflammation.[12] In previous studies, this inhibition has been shown to mediate cardioprotection in mice that overexpressed $P2X_4$, preserve function, and improve survival.[13,14] Additionally, in a murine model of heart failure, treatment with MRS2339, a $P2X_4$ non-hydrolysable agonist, conferred protection from heart failure.[15] In the human left ventricle, these purine receptors express mRNA. In the human right atrium and sinoatrial node tissue, the most expressed ionotropic purinergic receptor is $P2X_4$ followed by $P2X_7$, with notably diminished amounts of P2X 1, 2, 3, and 5.[12,16] Endothelial nitric oxide synthase, a result of $P2X_4$ activation, is a possible interacting protein activated by both calcium and calmodulin.[13] These observations suggest a potential utility for ATP administration as preconditioning therapy.

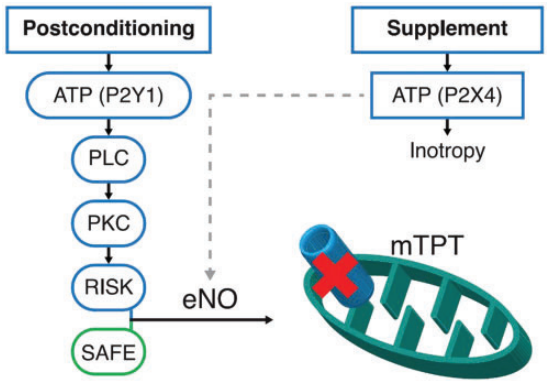

Figure 1. Two strategies of ATP as postischemic therapy. We targeted different ATP receptors using a cardioprotective postconditioning bolus (addressing the P2Y1 receptor) and regular cardiosupportive supplement therapy (triggering inotropic action via the P2X4 receptor). ATP: adenosine triphosphate; eNO: endothelial nitric oxide; mPTP: mitochondrial permeability transition pore; PKC: protein kinase C; PLC: phospholipase C; RISK: reperfusion injury salvage kinase; SAFE: survivor activating factor enhancement.

Moreover, research has stimulated continued interest in the clinical application and use of ATP as a cardioprotective agent that targets different receptors and signaling pathways, as reviewed by Sommerschild *et al*.[17] To our knowledge, there are no current clinical studies evaluating ATP for cardioprotection. In the current study, we used an isolated swine heart four-chamber working model as our experimental paradigm to investigate the potential benefits of ATP administration as a post-ischemic therapy (Figure 1).[3] For nearly two decades, the Visible Heart® Laboratories have employed these methodologies to investigate the delivery of various pharmacologic agents and/or to test various devices.[18] Using the same methodologies, we have also reanimated 83 human hearts, hence any beneficial cardioprotective agents found in our current swine model studies could potentially be applied to recovered human hearts and/or the use of *ex vivo* perfusion devices for extending the recovery period prior to transplantation.[19]

Materials and methods

This research was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Minnesota, and followed principles in the Guide for the Care and Use of Laboratory Animals.

Yorkshire swine (70–90 kg, 6–9 months of age) were initially sedated and anesthetized with an intramuscular

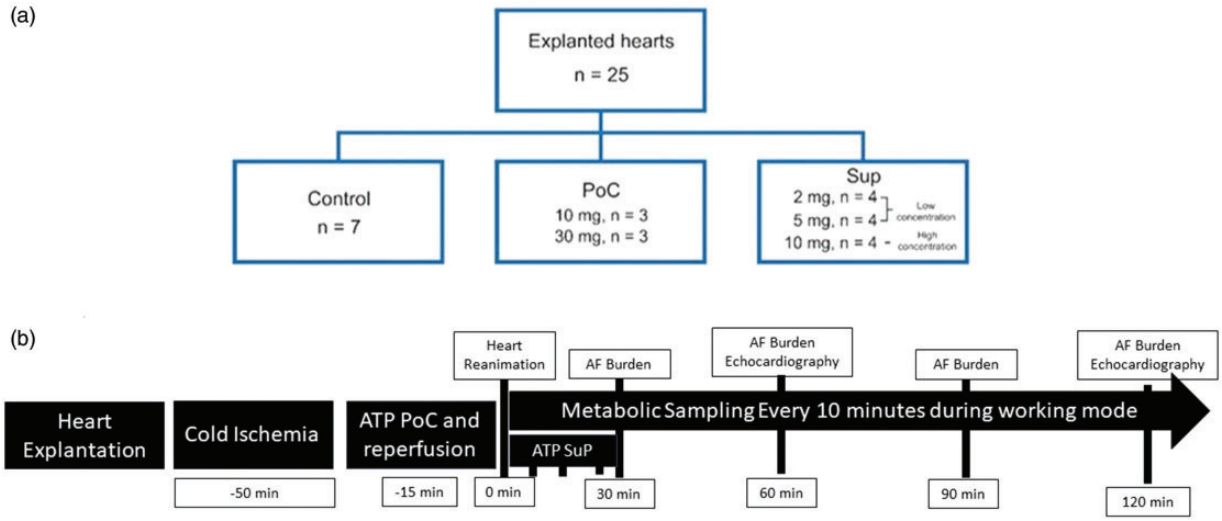

Figure 2. Experimental protocol. (a) Cohorts: We included a total of 25 explanted swine hearts in our analysis. (b) Timeline: We collected hemodynamic and metabolic data at various time points, as shown. AF: atrial fibrillation; ATP: adenosine triphosphate; PoC: postconditioning; RSW: right-side working mode; Sup: supplementary therapy; WM: four-chamber working mode.

injection of Telazol®, followed by intravenous injections of methohexital (5–7 mg/kg). Animals (n = 29) were intubated and anesthesia was subsequently maintained with isoflurane at >1.2 minimal alveolar concentration (MAC); real-time pressure and blood gas monitoring were employed as previously described.[18] Cardiac function was quickly stopped via cold modified St. Thomas cardioplegia administration (within minutes) and hearts were then explanted.[18–20] Each heart was then placed in a cold modified Krebs–Henseleit buffer solution (3–8°C)[21] and all great vessels were cannulated.[22]

Next, we set up each heart in the Visible Heart® *ex vivo* perfusion apparatus.[18,23] From explantation to apparatus connection, the preparation period averaged approximately 50 min. We began rewarming and reoxygenating (95%O_2, 5%CO_2) hearts over a period of 15–30 min, until they reached a temperature of 35.5°C. For experimental consistency, we then changed approximately 80–90% of the buffer solution. Each heart was instrumented for hemodynamic and electrical monitoring as previously described.[22] Once the heart temperature reached 36.5 ± 0.5°C, we delivered a 34J defibrillation shock to restore native sinus rhythm,[22] which marked the beginning of our *in vitro* experimental protocol. Hearts were placed into four-chamber working mode and then alternated periodically to a right-side-only mode (see Figure 2 for protocol details).[22]

Ex vivo protocols

For the 2 h *ex vivo* study periods, we compared three treatment groups (Figure 2): (1) bolus PoC group; (2) supplementary (Sup) group; and (3) control group. For the PoC group (n = 6), we administered two boluses of ATP to the reperfusion solution prior to defibrillation to assess its relative role as a sustained conditioning agent (10 mg: 3.5 μmol, n = 3; and 30 mg: 10.75 μmol, n = 3). In the Sup group (n = 12), we delivered regular dosages of ATP throughout the 2 h experimental period to assess its potential role as an acute pharmaceutical cardiosupportive agent; at 10 min intervals, we added 1 of 3 different dosages to the buffer solution: 2 mg (n = 4), 5 mg (n = 4), or 10 mg (n = 4). For the control group (n = 7), no drug was added to the buffer solution. The buffer within the apparatus was replenished after the 70 min time point (full buffer change).

Functional analyses of reanimated hearts

For functional analyses *in vitro*, our primary hemodynamic parameter was the maintenance of left ventricular (LV) pressure in the four-chamber working model. This was obtained via a pressure catheter placed in the LV and data were recorded. It should be noted that within a given 3 min interval during working mode, the LV pressure gradually decreased. Therefore, in these experiments, we chose to report the median value of the mean differential pressure (MDP), calculated by subtracting the diastolic pressure from the systolic pressure. The median value (unlike the mean) is robust to outliers, and MDP normally produces consistent results regardless of baseline drift. We also determined diastolic relaxation (Tau), rate of rise in LV pressure (dPmax/dt), and raw systolic and diastolic pressures.

Additionally, at the beginning, middle, and end of the *ex vivo* protocol, we used four-chamber and short-axis echocardiography to assess relative ejection fraction and LV wall thickness. As previously reported, throughout the *ex vivo* protocol, we used a Grass stimulator (Grass Medical Instruments, Quincy, MA, USA) and left atrial plunge electrode to assess arrhythmic effects of ATP.[22] Every 30 min, 1 ms pulses (50 Hz for 2 s) were applied to hearts; the duration of sustained atrial fibrillation (AF) after acquisition of a baseline threshold helped to determine the propensity for arrhythmia. We defined 1 min or more of AF as sustained AF.[22] If sustained AF was reached within five shocks, AF was allowed to continue for a maximum of 10 min, before the heart was defibrillated with 5J back into sinus rhythm.

Throughout the *in vitro* protocol, we used Radiometer ABL90 equipment (Radiometer America Inc., Brea, CA,

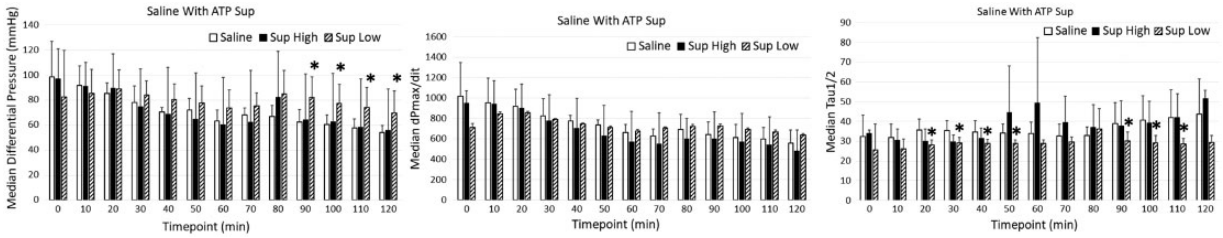

Figure 3. Postconditioning (PoC) hemodynamic parameters. Using a four-chamber working model, we measured the effects of ATP postconditioning on left ventricular differential pressure (*left*), the rate of rise in left ventricular pressure (*middle*), and diastolic relaxation (*right*) at various time points. *Statistically significant *P* (versus control group). ATP: adenosine triphosphate; dPmax/dt: rate of rise in left ventricular pressure.

USA) to measure venous and arterial blood gas samples every 10 min (Figure 2).

Statistical analyses

To determine statistically significant differences between groups, we used two-sided *t* tests. We also performed a mixed linear model to evaluate if there were any differences between groups and group effects. Secondly, a time point variance analysis, including *post hoc* comparison/Bonferroni correction, was performed. We controlled for the following groups: Sup ATP low dose (2 mg + 5 mg) and PoC (grouped 10 mg and 30 mg). Parameters included MDP, Tau, dpdt, venous pO_2, and venous lactate. A P value <0.05 was considered to indicate significance, unless otherwise noted. The displayed graphs show median values ± STD.

Results

After completing 29 swine experiments for this study, 4 were excluded due to protocol irregularities unrelated to administered treatments; 2 reanimated hearts elicited excessive arrhythmia which necessitated off protocol intervention, and 2 others became hypotensive *in situ* or *in vitro*. Although we used the remaining 25 swine for our analyses, the complexity of this protocol and malfunctioning data collection systems sometimes limited our ability to include every parameter for every time point, for each comparison. Therefore, for each parameter reported, we indicated the final sample size; a selection of recorded values can be found in the electronic supplementary materials.

After a minimum of 3 h on the *ex vivo* perfusion apparatus, hearts increased in mass; average heart weight was 423.2 g (SD 47.2) at baseline and 645.4 g (SD 84.6) at the end of the experiment. This was due to edema caused by the relative lack of oncotic pressure regulation induced by the buffer solution. Yet, these weight gains did not differ significantly between groups (data are available in supplemental materials). Additionally, these findings were consistent with our echocardiographic measurements of LV thickness, which increased after the first hour by 17% in the control group, 14% in the PoC group, and 31% in the Sup group. After the second hour, LV thickness increased (as compared with baseline measurements) by 36% in the PoC group and 37% in the Sup group (we did not collect data after the second hour in the control group). It may also be noted that an increase of time on the apparatus past this study would result in increased edema and may not reflect the weight gained during the study period, however no statistically significant differences in weight were identified between groups.

ATP as a PoC agent

In the PoC groups, hearts elicited a significantly decreased MDP as compared with the control group (Figure 3). This observation remained significant throughout the duration of the protocol for both the 10 mg and 30 mg PoC dosing subgroups. Yet, the extent of LV functional depression appeared to be dose dependent; MDP was lower with 30 mg than with 10 mg (for both groups $P < 0.05$ after 90 min). These effects were not statistically significant within the two PoC subgroups after 2 h *ex vivo*.

We found no evidence of functional stabilization of hemodynamic parameters after the 70 min full buffer change, perhaps implicating an acute reaction to ATP within this paradigm. Note that the PoC groups did not differ from the control group in terms of Tau, dPmax/dt, or normalized contractility measurements.

Our measurements of arrhythmogenicity were highly variable. At the 30 min point, the average cumulative duration of AF was 31 s in the control group, compared with 307 s in the 10 mg PoC subgroup and 264 s in the 30 mg PoC subgroup. At the 90 min point, the cumulative duration of AF was 89 s in the 30 mg PoC subgroup.

Our metabolic measurements followed expected physiologic trends, showing increases in lactate level and gradual decreases in glucose concentration over time (supplemental data available). We chose both lactate and partial pressure of oxygen (pO_2) for more detailed analyses; overall, both parameters tended to be lower in the PoC group than the control group. Although the PoC group elicited poorer hemodynamic function than the control group, the PoC groups' lactate levels at the 120 min point were significantly lower ($P = 0.05$).

ATP as a Sup agent

In contrast to the PoC group, the Sup group elicited gradually improved performance compared to the control group (Figure 4). Based on trends, we identified that the dosages could be separated into low (2 mg and 5 mg) and high (10 mg) concentrations. The low concentrations were superior to the high; the most pronounced differences between the low concentration Sup group and control

group was in MDP, at the 90 min point ($P = 0.02$). The beneficial effect remained significant through the 120 min point ($P = 0.05$).

We noted no significant differences in dPmax/dt values between the low concentration Sup subgroup and control group at any time point. However, the low concentration Sup subgroup generally elicited lower Tau values, indicating improved diastolic relaxation ($P < 0.05$; Figure 4).

Arrhythmia induction in the Sup group elicited only minor deviations from the control group. At the 90 min point, arrhythmias were prolonged in the low concentration Sup subgroup, with an average of 219 s. It was also prolonged in the high concentration Sup subgroup, with a 77 s average.

Changes in hemodynamic function in the low concentration Sup subgroup were consistent with elicited metabolic responses, with a significantly smaller increase in lactate levels over time as compared with the control group. These differences continued through the 120 min point ($P = 0.01$). For both Sup subgroups, venous pO_2 remained lower than for controls, with the most pronounced difference occurring at the 120 min point ($P = 0.05$; Figure 5).

Discussion

In this study employing an isolated swine heart model, we demonstrated positive effects of ATP administration in this ischemic transplant scenario. We investigated different administered concentrations and timing protocols as a means to account for diverse possibilities for the proposed underlying mechanisms of action. Our overall goal was to mitigate reperfusion injury of these isolated large mammalian hearts and reinforce their ability to function in a simulated transplant scenario. We reanimated hearts using Visible Heart® methodologies on an *ex vivo* perfusion device, after a hypothermic ischemic period of only 45–50 min.

The first hypothesis we sought to test was that the effects of ATP administration would be acute and occur only at the initial point of reperfusion. To do so, we employed the PoC paradigm via administration of bolus doses of ATP at the start of the *ex vivo* protocol prior to reanimation in a native sinus rhythm. We observed that single ATP bolus administration elicited dose-dependent depressant effects on cardiac function within that paradigm. This could indicate that these concentrations of ATP are toxic during this initial reperfusion phase, or that these isolated hearts enter some sort of beneficial state of lower hemodynamic/metabolic output (hibernation) that does not recover within our experimental time frame. In other words, this decreased function could have benefits, including preserving metabolic reserves and decreasing vascular damage. Such benefits would presumably lead to a point where treated hearts could recover and have better function.

The second hypothesis tested was that ATP functions as a cardiosupportive agent when administered in an *ex vivo* perfusion setting. We tested this via the incremental addition of ATP at a given concentration before each working

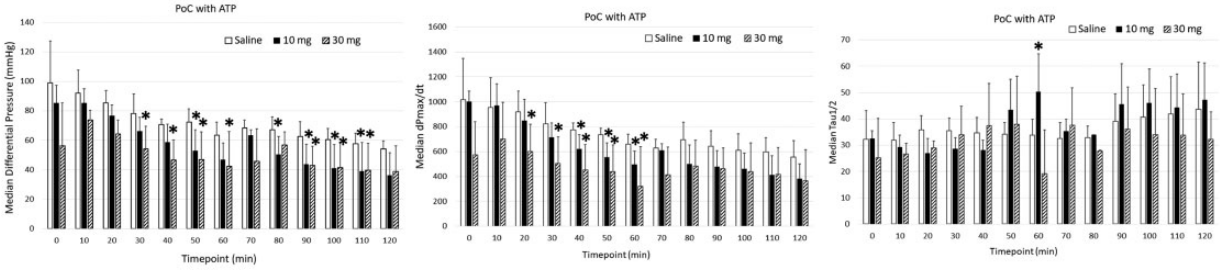

Figure 4. Supplement therapy (Sup) hemodynamic parameters. Using a four-chamber working model, we measured the effects of ATP as a regular cardiosupportive supplement therapy on left ventricular differential pressure (*left*), the rate of rise in left ventricular pressure (*middle*), and diastolic relaxation (*right*) at various time points. *Statistically significant *P* (versus control group). ATP: adenosine triphosphate; dPmax/dt: rate of rise in left ventricular pressure.

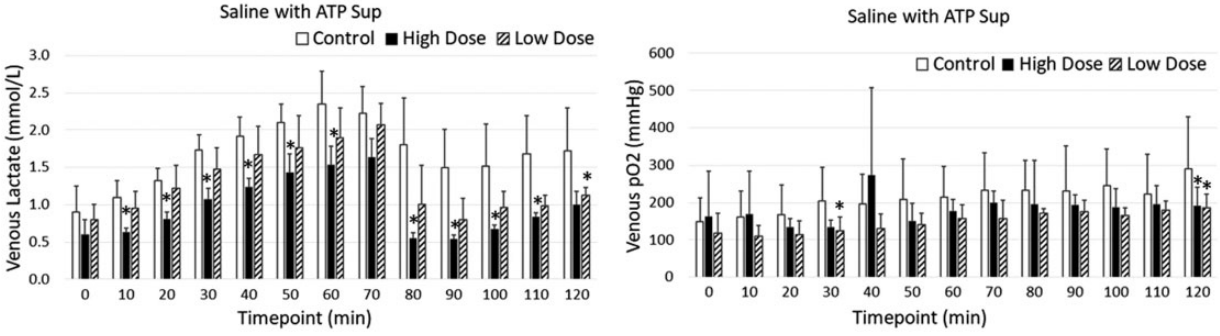

Figure 5. Supplement therapy (Sup) venous lactate and pO_2. Using a four-chamber working model, we measured the effects of ATP as a regular cardiosupportive supplement therapy on venous lactate levels (*left*) and pO_2 (*right*) at various time points. *Statistically significant *P* (versus control group). ATP: adenosine triphosphate; pO_2: partial pressure of oxygen.

model. However, it should be noted that at the 70 min buffer change, we made the major assumption that minimal amounts of the previous ATP were utilized within the first half of the experiment, therefore we again added back the determined accumulated concentrations after the buffer change. On one hand, these upper concentration bounds can be used to make inferences about the relative toxicity or benefits of providing ATP concentrations to the buffers over time. Interestingly, these high doses of ATP, mid-experiment, did not negatively affect performance of these hearts after buffer changes (as compared with the control group). This lack of effects implies that: (1) ATP and/or its byproducts continued to be important for cardiac maintenance; (2) these hearts were not vulnerable to exposure in this phase as during reperfusion; or (3) the presence of ATP was irrelevant and its beneficial effects had already subsided. Nevertheless, taken together our observations implicate that ATP administration works most effectively as a cardiosupportive agent, in our global ischemic transplant scenario.

In the Sup experimental protocol, we observed both functional and contractile improvement with the sequential administration of ATP to the reperfusion buffer solution, every 10 min. These improvements, which were most pronounced at the 90 min time point, suggest a therapeutic role for ATP as an inotropic, extracellular signaling agent targeting various receptors. We further observed that $P2X_4$ activation might be one of the pathways associated with elevated levels of endogenous extracellular ATP, which in turn induces protective effects to attenuate reperfusion injury. The varied responses to ATP administration also suggest that it has complex modes of interaction with the underlying biology. Perhaps this is not surprising, given the prominence of ATP as a ubiquitous energy-storing molecule. Nevertheless, the advantages of ATP having diverse targets must be weighed with the potential negative effects on various pathways. Importantly, we observed that regular cardiosupportive administration of ATP was associated with increased LV pressures, lowered lactate levels, and decreased venous oxygen levels. Again, this was in marked contrast to the single bolus administration of ATP in the PoC, which decreased functional values (MDP, Tau) throughout the experiments.

We consider that both treatment strategies, if appropriately administered, may elicit value in various clinical transplant scenarios, yet the relative administered dosages and/or application times may differ from those utilized here. For any type of *ex vivo* experimental protocol, it is advantageous to maintain functional viability (e.g., for device testing). On the other hand, this may not necessarily be the case for clinical applications, if this added function leads to long-term cell death. Therefore, future investigations are required to determine which experimental outcomes might be most beneficial for clinical practice. If one considers that both strategies of ATP administration utilized here are beneficial, it is important to explore the question: Can both be used in the same paradigm for added benefits? In general, our data suggest the potential to apply the Sup strategy to an *ex vivo* heart perfusion scenario, which technological advancements could soon make a reality. Yet, we understand that additional cellular and molecular data will be required to support these functional analyses.

More specifically, for the low concentration Sup subgroup, the minimization of induced arrhythmias was most pronounced at the 90 min point, when the concentration of ATP in solution was comparatively high, after buffer changes and the subsequent bolus administration. Such protective behaviors have been supported by previous reports describing a high concentration of ATP to be arrhythmogenic.[12] Therefore, if bolus ATP administration is used for human transplant recipients, an appropriate anti-arrhythmogenic agent could be used in tandem for therapeutic benefits; these applications will require future research.

Our data may also suggest that there are interactions of different ATP receptors and signaling pathways, likely both dose and time dependent. In the Sup group, because we started ATP administration shortly after reperfusion, PoC pathways could have also been activated. On the other hand, the very low doses of ATP circulating during reperfusion might not have negative effects on hemodynamic function, but may actually be cardioprotective to the mPTP, but utilizing different mechanisms of action simultaneously.

Limitations

Molecularly, in the context of pre- and postconditioning of the heart, signalizing pathways of swine hearts likely deviate to some degree from those of human hearts. For example, the RISK pathway, deemed key to cardioprotection, has been reported to be underexpressed in pigs. Still, Musiolik *et al.* demonstrated that PoC effects were achieved in a swine model even though the RISK pathway was pharmacologically blocked, indicating that other mechanisms must be involved in ultimately inhibiting mPTP. Such mechanisms could include reducing both reactive oxygen species (ROS) and calcium overload, thereby improving maintenance of acidosis during early reperfusion.[24] Similarly, the survivor activating factor enhancement (SAFE) pathway, which is considered to be involved in signal transduction of tumor necrosis factor-alpha (TNF-a) through sphingosine kinase and STAT3, could be the primary PoC pathway. Both pathways may interact, perhaps even converge, at the same target, namely the mitochondria. Future molecular investigations are needed.[25]

In the present study, we did not analyze the relative buffer concentrations of ATP or its breakdown products. Preliminary unpublished data from our laboratory have shown that ATP, given after an extended *ex vivo* period even in high concentrations, did not have a negative effect on cardiac function. Further, after the administration of a bolus dose after buffer change, we did not observe any negative impact on hemodynamic measurements.

Another aspect of the present study is that we used a clear acellular buffer which we routinely employ to take endoscopic internal images (Visible Heart® methodologies). This buffer may be considered as less physiologic than utilizing whole blood. Moreover, our protocol

involved a relatively short study period, and longer applications of ATP administration, where global ischemia may be ongoing, need to be studied.

Future directions and clinical application

Perhaps the aforementioned multiplicity in the pharmacologic mechanisms of action attributed to ATP administration is consistent with the contrasting results observed with the two bolus strategies of ATP we tested. Yet, future studies with larger sample sizes will be required to elucidate these mechanisms of action; one should additionally employ various cardiac models of varied cellular biology and/or human studies.

Both of our strategies for ATP administration in the reanimated swine hearts could be of mechanistic interest given emerging *ex vivo* technologies, particularly the assessment of *ex vivo* cardiac function before transplantation. For example, several recent clinical trials have focused on use of the Organ Care System™ (OCS™; TransMedics, Inc., Andover, MA, USA) to extend the period of time between organ recovery and transplantation.[26,27] This portable perfusion and monitoring system maintains donor hearts in a near-physiologic, metabolically active, and functioning state *ex vivo*. Perfused with warm, oxygenated, nutrient-enriched buffer (e.g., blood), the heart is kept in a functional state until ready for transplantation. It is considered that by using molecular modulators (e.g., ATP) one might be able to significantly reduce the ischemic state of hearts on an OCS™, resulting in increased numbers of viable organs available for waiting transplant candidates.

Yet, unanswered questions from our study underscore the need for more research to identify the appropriate dosages of ATP for translational use. For example, important barriers to successful clinical introduction of ATP administration include the downregulation of RISK pathways and the varied pharmacologic half-life values of ATP, which may exist in an oxygenated buffer solution versus a blood-traversing complex vasculature.

One final consideration for the future application of ATP administration is that at high extracellular concentrations, ATP may be pro-arrhythmogenic or could even cause atrioventricular blockage.[16] Furthermore, ecto-ATPases cause rapid ATP breakdown, so metabolite effects such as AMP or adenosine require future attention. ATP is also a known vasodilator and contributes to a state of hypotonicity, thus future studies may identify optimal dosaging, potentially combined with another agent, to mitigate side effects or enhance the therapeutic impact of ATP.

In summary, we employed an isolated swine heart four-chamber working model to investigate two potential strategies for ATP administration as an *ex vivo* therapy: (1) the application of a single bolus dose during reperfusion (PoC), and (2) repeated bolus dosing throughout the experiment (Sup). *Ex vivo* swine hearts in the Sup group elicited significantly higher LV function during the 2 h experimental monitoring period. In contrast, ATP administration in the PoC group appeared to induce a degree of depressed hemodynamic function. These data suggest varied functional roles of ATP administration relative to their use in *ex vivo* perfusion strategies, yet additional investigations and optimizations are needed.

Author contributions: MSS: Experiment design, data collection/interpretation, report writing, manuscript approval; ENG: Data collection/interpretation, report writing, manuscript approval; TLI/LMM/ARM/MMS: Data collection, manuscript approval; RCBD: Critical review, manuscript approval; PAI: Experiment design, data collection, critical review, manuscript approval.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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