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# Singlet Oxygen Energy Illumination during Ischemia Preserves High-Energy Phosphates in a Concordant Heart Xenotransplantation Model

**D. J. Lukes[1, 2], A. Lundgren[1, 2], J. Wilton[1, 2], A. Lindgård[2, 3], E. Omerovic[2], O. Rakotonirainy[2, 3], A. Karlsson-Parra[4], M. Olausson[1, 2], and B. Soussi[2, 3, *]**

[1] *Department of Surgery and Transplantation*
[2] *Wallenberg Laboratory for Cardiovascular Research*
[3] *Bioenergetics Group and Lundberg Center for Bioanalysis*
[4] *Department of Clinical Immunology*
*Sahlgrenska University Hospital and Göteborg University, Göteborg, Sweden*
*e-mail: soussi@wlab.gu.se
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**Abstract—Introduction:** We have previously demonstrated that illumination with singlet oxygen energy (SOE) could reduce the generation of reactive oxygen species (ROS) *in vitro*. We have here investigated whether SOE illumination induced during ischemia could preserve high-energy-phosphate (HEP) levels in an *in vivo* xenotransplantation heart model.

**Material and methods:** Cervical transplantations between inbred Lewis (RT1[1]) rats (recipients) and Golden–Syrian hamsters were performed and followed with daily *in vivo* $^{31}$Phosphorus Magnetic Resonance Spectroscopy ($^{31}$P MRS) over 4 days. The phosphocreatine (PCr) to beta adenosine triphosphate (β-ATP) ratio was calculated. The recipients were randomized to any of 2 groups (grp.): illumination of the NaCl and the grafts before reperfusion (r.p.) (grp. A, $n = 7$) illumination of the NaCl used during explantation (grp. B, $n = 8$). SOE was produced as photons at λ 634 nm with the Valkion® equipment, and the grafts were illuminated for 10 min before the onset of reperfusion. The cold ischemia time (+4°C) was standardized to 30 min.

**Results:** The PCr/β-ATP ratio of the illuminated grafts on day 1 was 39% higher ($p = 0.002$ vs. Grp. 1; 1.99 ± 0.12 vs. 1.43 ± 0.08; mean ± SEM) and similar to the baseline values *in situ*, 1.80 ± 0.08.

**Conclusions:** We demonstrate for the first time *in vivo* that SOE illumination, when induced in xenografts before r.p., can preserve the energetic status of concordant hamster grafts and counteract the metabolic effects of short-term ischemia. It remains to be investigated whether reduction of early ischemic events in experimental xenotransplantation, for instance with SOE, has any impact on the survival and future development of chronic rejection in immunosuppressed recipients of xenografts.

## INTRODUCTION

The use of photon energy such as low-power lasers, visible to IR light, is well established in biology and is increasingly used in medicine [1–3]. Singlet oxygen $O_2(^1\Delta_g)$ can be produced photochemically by energy transfer from an excited photosensitizer [4]. The energy emitted from singlet oxygen upon relaxation to its triplet ground state $O_2(^3\Sigma_g^-)$ is captured as photons at 634 nm and here referred to as singlet oxygen energy (SOE). We have previously demonstrated in *in vitro* experiments that treatment with SOE decreased the generation of reactive oxygen species (ROS) in human monocytes by up to 60% [5]. In a recent experiment on a rat hindlimb ischemia model, we were able to demonstrate *in vivo* that SOE illumination before, during, and after reperfusion improved the bioenergetic recovery of ischemic rat rectus femoris muscle measured as the high-energy phosphates (HEP), phosphocreatine (PCr), and beta Adenosine Triphosphate (β-ATP) using *in vivo* $^{31}$Phosphorus Magnetic Resonance Spectroscopy (*in vivo* $^{31}$P MRS) [6]. Chronic rejection in allografts, in heart transplants due to coronary arteriosclerosis, prevents allotransplantation from becoming the ultimate solution to terminal end stage disease and is now the major factor limiting long-term survival [7–9]. Xenotransplantation, the transplantation of organs and tissue between species, bears the potential of providing an unlimited access to organs, both as a bridge to allografts (transplanted organs within a species) and as a permanent solution [10].

Early ischemic events may contribute to later rejection episodes [11] and the development of chronic rejection features in allografts [12]. The exact mechanisms are not fully elucidated but involve endothelial activation, up-regulation of adhesion molecules, and activation of the clotting cascade [12]. Long time surviving (LTS) xenografts, where the humoral barrier is crossed, also display to some extent features resem-

bling chronic rejection in allografts [13, 14]. However, the importance of the ischemia-reperfusion insult in xenotransplantation has not been addressed.

Reactive oxygen species (ROS) are highly toxic oxidants generated during inflammation and postischemic reperfusion and can cause significant tissue injury [12]. Furthermore, the formation of oxygen free radicals during reperfusion has been proposed to quench available nitric oxide (NO) and cause a failure in the NO pathway during preservation/transplantation thereby, most likely, reducing the blood flow to the reperfused grafts [15].

We have recently provided *in vivo* $^{31}$P MRS evidence for a correlation between decreasing ratios of HEP and acute xenograft rejection in the concordant mouse-to-rat model [16]. The aim of the present study was to investigate whether exposure of hamster hearts to SOE through photon illumination at $\lambda$ 634 nm at various times during and after the transplantation procedure could improve the cellular energetic status followed by *in vivo* $^{31}$P MRS. This study provides for the first time *in vivo* evidence for the capability of SOE to improve the status of HEP in hamster xeno hearts if transferred before the onset of reperfusion in the rat recipient.

## MATERIALS AND METHODS

### *Animals*

Male inbred Lewis (RT1$^1$) rats (purchased from Mollegaard, Skensved, Denmark) weighing 200–220 g were used as recipients, and male Golden Syrian Hamsters weighing 70–80 g acted as donors (purchased from B&K farm, Sollentuna, Sweden). The animals were allowed to settle in the animal quarters for several days before any experiments were started and had free access to standard food pellets and water. The experimental protocol was reviewed and approved by the Ethics Committee of the University of Goteborg (Goteborg, Sweden). The investigation conforms to the Guide for Care and Use of Laboratory Animals (NIH publication 85–23, revised 1985).

### *Transplantation Procedure*

Briefly, the animals were anesthetized with a combination of Hypnorm® (Fentanyl; 0.6 ml/kg) and Stesolid® (Diazepam; 2.5 mg/kg) and given i.p. Thereafter, Temgesic® (Buprenorhine) was administered in a dose corresponding to 0.01–0.05 mg/kg. During the operative procedure, a constant body temperature (37° ± 0.5 °C) was maintained by a specially adapted homoeothermic blanket system (Harvard Apparatus) consisting of a warming pad and a rectal probe for the regulation of the heat output. After the initialization of the cooling of the hearts in the donor animals (see below), the system was switched off. The same system was used for the recipient operation. The donor thorax was opened, the inferior caval vein perfused with cold (+4 °C) saline solution (NaCl, 0.9%, Braun Medical AB, Solna, Sweden) with Heparin® (AB Kabi, Stockholm, Sweden) at a 10 : 1 dilution, and then the superior caval vein was ligated. Thereafter, the inferior caval vein was perfused with cold saline solution and ligated, and the heart was excised with a last perfusion in the aortic arch before the pulmonary veins were ligated. The recipients right common carotic artery and jugular vein were prepared with "cuffs," as earlier described, and the heart grafts connected using ligatures around the prepared "cuffs" [17]. The ischemia time outside the donor (here defined as the time between excision of the heart and the onset of circulation) did not exceed 10 min in any of the study groups (see below). Note that the total ischemia time counted from the onset of explantation did not exceed 30 min in any of the groups or experiments. Meanwhile, the hearts were stored in cold (+4 °C) saline solution (NaCl, 0.9%, Braun Medical AB, Solna, Sweden). Rejection was defined as loss of regular palpable contractions and visual activity.

### *Treatment with Singlet Oxygen Energy (SOE)*

SOE was produced with the Valkion® equipment (Polyvalk AB, Sweden) as photons via a fiber-optic cable (cable length 142 cm; diameter of the end of the fiber-optic cable 3 mm). In the Valkion® equipment, singlet oxygen was generated through a photosensitization process, using a phtalocyanine zinc (II) blue reddish dye as photosensitizer [4]. This sensitizer is one of the few that can perform in gaseous atmosphere [4]. Furthermore, it has a good heat and light resistance and can be applied on metal surfaces. As a light source, 6 light-emitting diodes (LEDs) were used. There are different techniques to make a coating of the sensitizer on a metal surface. When using diodes as a light source, the heat developed during the process is much less than with the use of a halogen lamp, and consequently the requirements for the coating are also less severe. The activation chamber developed to produce the singlet oxygen consisted of an aluminum plate coated with the sensitizer. Air with a relative humidity of 90% was used as the medium for singlet-oxygen generation; the humidity was generated by circulating air through a water flask. The lifetime of singlet oxygen in this medium is about 2 μs. A seal between the aluminum plate and sensitizer prevented the activated air from escaping. SOE illumination was done directly on the hearts by positioning the end of the cable 5 mm from the hearts and illuminating it by slightly moving the cable end over the entire heart surface 10 min before the onset of reperfusion. Illumination of hamster hearts by SOE was performed according to the study protocol described below. In group A, the saline injected during explantation of the hearts (typically 3–4 cc) was illuminated by gently moving the cable end for 10 min in the little beaker used to store the NaCl. In experiment,

group 2 was illuminated each day for 10 min through the skin before the $^{31}$P MRS acquisition

### *Study Protocol*

**Experiment 1.** The explanted hearts were randomly allocated to either of the two groups: illumination of the NaCl and the grafts before reperfusion (r.p.) (group A, $n = 7$) and illumination of the NaCl used during explantation (group B, $n = 8$).

**Experiment 2.** In a second experiment, the grafts were either illuminated before (group 1, $n = 5$) or after the reperfusion (group 2, $n = 6$) on each subsequent day before the $^{31}$P MRS measurements until graft rejection. The data in each experiment were compared to baseline data obtained by measuring on 5 hamster hearts *in situ*.

### *Anesthesia during $^{31}$P MRS Measurements*

The transplanted rats were anaesthetized with a balanced gas anesthesia using a Titus NMR Portable Anesthetic Apparatus modified for use with scanners (Dräger Aktiengesellschaft, Lubeck, Germany). Isofluran (Forene®, Abbot, Kista-Stockholm) was used as anesthetic gas, $N_2O$ and $O_2$, as carrier gases. A homemade mask was designed to fit the nose of the rats with both inlet and outlet channels for incoming and exhaled gas. After initial induction, using high flows of Isofluoran and $N_2O/O_2$, the gases could be turned down to maintenance levels of about 1–1.5% Isofluoran and an approximately 70 : 30% ratio of $N_2O$ and $O_2$ corresponding to approximately 1.4–1.5 : 0.6–0.7 l/min. The rats fell quickly asleep and were observed for a minimum period of 5 min before the measurements started. During the measurements, the rats were regularly observed with respect to movements and breathing rate and pattern. After anesthesia, the rats woke up within 1–2 min and regained their normal behavior.

### *$^{31}$Phosphorus Magnetic Resonance Spectroscopy ($^{31}$P MRS)*

***In situ* volume-selected $^{31}$P MRS on hamster hearts.** In order to obtain baseline values on hamster hearts *in situ* before explantation and subsequent transplantation, we performed *in situ* volume-selected $^{31}$P MRS measurements. The method has previously been described in detail [18]. Briefly, MR imaging and spectroscopy were performed on a Bruker 24/30 Biospec System (Bruker, Rheinstetten, Germany) interfaced with a 2.35 T 20 cm horizontal bore magnet. The magnetic field was optimized by adjusting the current through shim coils and observing the $^1$H (water) signal from the tissue. The magnetic field homogeneity was accepted when the line width of the $H_2O$ signal was less than 50 Hz (0.5 p.p.m.). A $^1$H/$^{31}$P double tuned surface coil of 5 cm diameter was used for radio frequency transmission and reception. Continuous ECG signals were acquired (Pysiogard SM 785 MR monitoring system; Bruker, Karlsruhe, Germany) and used for synchronization of the radiofrequency (RF) pulses and for monitoring of the heart rates. A gradient echo method was used for visualization of the heart and selection of volume of interest (VOI). All images were obtained with synchronized RF pulses to the cardiac rhythm. Thereafter, localized shimming was performed on the VOI (typically $10 \times 12 \times 14$ mm (1.68 cm$^3$) including as much as possible of the left ventricle) using cardiac gated STEAM localization pulse sequence observing the proton ($^1$H) signal. The Cardiac gated image selected *in vivo* spectroscopy (ISIS) method [19] was employed for the volume-selected $^{31}$P spectroscopy. Acquisition parameters were 512 scans with a 4.5-s repetition time, 4 k data points and 2500 Hz sweep width giving a total scanning time of 38 min. A 4-ms adiabatic amplitude shaped pulse was used for inversion, and a 3.5-ms half sinus cosine pulse was used for excitation in the ISIS method. Spectroscopic processing consisted of exponential multiplication of acquired free-induction-decay (FID) line broadening of 6 Hz. Then the data was Fourier transformed, and first-order phase correction was applied manually. Areas of Pi, 2.3–DPG, PCr, and β-ATP were integrated with the Bruker routine. Proton decoupling was used, but we were unable to separate the 2.3–DPG signal from the Pi peak, which precluded the calculation of the PCr/Pi ratio and pH. Myocardial PCr/β-ATP was corrected for partial saturation and blood contamination as previously described [18].

***Ex situ* $^{31}$P MRS measurement on transplanted hearts.** The method has also been previously described [16]. Briefly, a 10 mm $^1$H/$^{31}$P double tuned surface coil was placed over the cervically transplanted xenograft in direct contact with the skin. The recipient rat was placed and fixed on a plastic rack in standard fashion to prevent displacement of the surface coil relative to the graft and then put into the magnet. A 90° pulse, with a 50 μs pulse width, was used to acquire the $^{31}$P MRS spectra with a repetition time of 2 s. For the calculation of saturation correction factors, a repetition time of 16 s was used. A total number of 2048 scans were acquired on each animal, with a total scanning time of 1 h 8 min. The spectroscopic processing was the same as described above for the *in situ* volume-selective $^{31}$P MRS method. $^1$H MRI (Magnetic Resonance Imaging) was also performed here to see the excitation profile of the surface coil and to control the VOI. These images showed that the spectra were derived from the transplanted hearts. The measurements started on the first postoperative day (d 1) and continued thereafter until one day post rejection (d 4). The MRS operator was blinded to the group identity of the animals and were only given randomly allocated numbers.

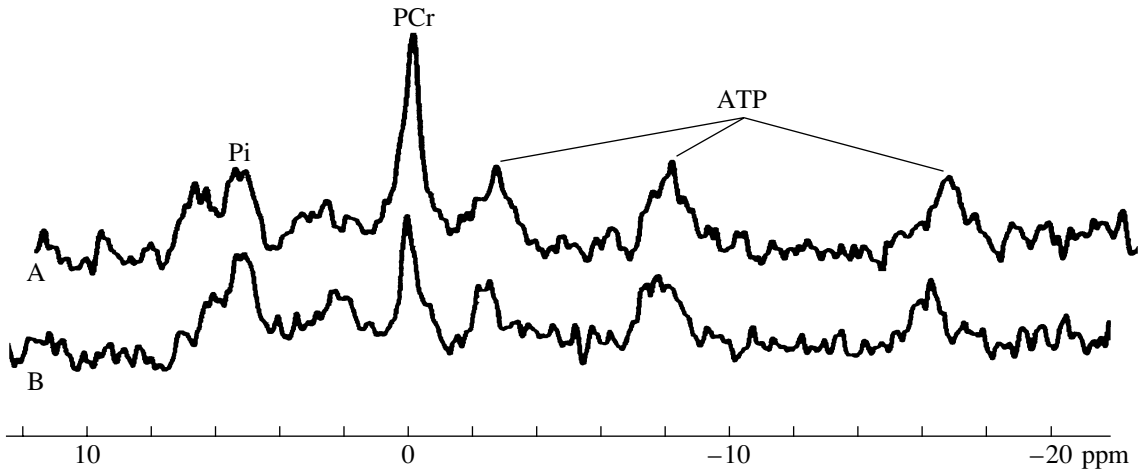


**Fig. 1.** Representative *in vivo* $^{31}$P MRS spectra on day one post transplantation of hamster hearts treated with singlet oxygen energy (SOE) during 10 min prior to reperfusion (group A) vs. control (group B). Pi: (inorganic phosphate); PCr: (phosphocreatine); ATP: (adenosine triphosphate); p.p.m. (parts per million). The PCr/β-ATP ratio is higher in the SOE treated group (1.99 ± 0.12 vs. 1.43 ± 0.08; $p$ = 0.002; independent t-test). Data presented as mean ± SEM.

### *Statistical Analysis*

The Kolmogorov–Smirnov test was used to assess the data for normal distribution. Since the data were normally distributed according to the test, the unpaired-students t-test was used to calculate intergroup differences on the respective postoperative days, and the paired t-test, within the groups. The data for graft survival were calculated as means ± SEM (Standard Error of the Mean). On the basis of the relative ratios for PCr/β-ATP for each animal on each postoperative day, the means ± SEM for that day were calculated. To compensate for multiple comparisons, a p value at or below 0.01 was required to consider a difference as significant. All statistical calculations were performed with the WinStat 3.1 statistical software for Windows (Kalmia Co. Inc., Cambridge, MA, USA).

## RESULTS

### *Graft Survival*

The mean graft survival was 3.0 ± 0 days in both groups in both experiments ($n$ = 26). No difference was seen between the various treatment groups in this respect.

### *In vivo $^{31}$P MRS*

**Experiment 1.** Representative $^{31}$P MRS spectra from a transplanted hamster heart on day one in the group subjected to SOE illumination of the grafts prior to reperfusion (group A) and in the control group (group B) are shown in Fig. 1.

The PCr/β-ATP-ratios of each group on each postoperative day together with the average baseline *in situ* value are depicted in Fig. 2. The PCr/β-ATP ratios of group A in experiment 1 differed significantly ($p$ = 0.002) on postoperative day 1 from control group B (illumination of the NaCl), and the PCr/β-ATP level was 39% higher than in the control group 1.99 ± 0.12 vs. 1.43 ± 0.08. No significant difference was seen against the *in situ* value, 1.8 ± 0.084. The ratio remained higher thereafter, although not significantly different compared to group B. The group-B level on day 1 was, however, significantly lower then the *in situ* value ($p$ = 0.007).

**Experiment 2.** The group-1 ratios (1.94 ± 0.16) were not different from the *in situ* value were as the group 2 values did (1.40 ± 0.11), but were not different from the data of control group A in experiment 1. The

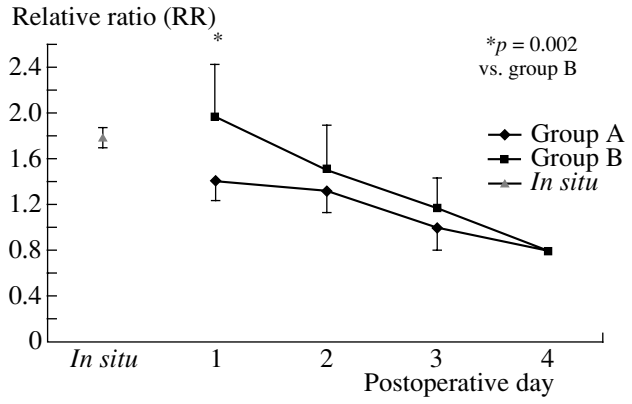


**Fig. 2.** This graph shows the gradual decline in the relative ratio (RR) of phosphocreatine (PCr) to beta adenosine triphosphate (β-ATP) on each postoperative day in hamster hearts transplanted to rat recipients as measured with *in vivo* $^{31}$P MRS. Group A = illumination of the NaCL used during explantation and of the hearts before r.p. with singlet oxygen photons at λ 634 nm (10 min) ($n$ = 7). Group B = control ($n$ = 8). *$p$ = 0.002 vs. control; independent t-test). r.p. = reperfusion. Data presented as mean ± SEM.

difference here also emerged on day 1 ($p = 0.009$). The data are not shown in a separate graph or table.

## DISCUSSION

This study provides for the first time *in vivo* evidence of the capability of SOE transferred as photons at a wavelength of 634 nm to preserve the levels of HEP, measured as PCr and ATP continuously and noninvasively by *in vivo* $^{31}$P MRS, in transplanted hamster xeno hearts. This technique has proven to be a valuable tool for studying the physiology of transplanted hearts and the effect of different therapies [20–25]. The correlation between high-energy phosphates and rejection score in experimental allotransplantation models has been good, and decreasing levels of high-energy phosphates have been shown to have an over 90% sensitivity, specificity, and predictive value for the subsequent day's biopsy-verified rejection [26]. Recently, we also provided evidence also for a correlation between falling ratios of HEP and progressive xenografts rejection in the concordant mouse-to-rat xenotransplantation heart model [16]. A correlation between HEP and histological findings of chronic rejection in a heterotopic allotransplantation heart model has also been demonstrated [27].

The effect of SOE in this study was only seen in the group where illumination took place before the onset of reperfusion. If illumination took part afterwards on subsequent days no effect could be demonstrated. The difference emerged statistically on postoperative day 1, and the differences became smaller thereafter, although the energy levels in this group were higher throughout the study when compared to the control group (Fig. 2). The mean PCr/β-ATP ratio, an index of the balance between energy production and utilization, i.e., an indicator of cellular phosphorylation potential [28], of the grafts that were illuminated with SOE photons during ischemia ($1.99 \pm 0.16$) was comparable to what has been reported as the steady-state level of rat hearts *in situ* and that we obtained in the hamster hearts in this study [29, 30]. Thus, the energetic balance of the illuminated hamster hearts where SOE was induced during ischemia was preserved compared to the control group and *in situ* hearts.

Singlet oxygen $O_2$ ($^1\Delta_g$) is generated when "regular" triplet oxygen ($O_2$) is excited to a higher energy state by photochemical energy transfer from an excited photosensitizer [5]. This form of oxygen is a known harmful species in biological systems [31]. However, when relaxing to its triplet ground state, the emitted energy, SOE, can act beneficially and has been shown in a recent publication from our group to reduce the production of ROS by up to 60% in an *in vitro* system using isolated human monocytes [5]. This effect seems to be mainly mediated through the inhibition of NADPH oxidase, the major source of superoxide anions ($\cdot O_2^-$) [5]. We know from *in vitro* studies that low doses of photon energy can modify cell behavior, whereas high doses are cytotoxic [32]. For SOE, the dose response *in vitro* occurs in a narrow dose range [5].

It is generally accepted today that, during ischemia, free-radical-generating reactions take place in the absence of oxygen and that the burst of free-radical production leading to oxidative stress occurs mainly during the reperfusion phase [33–36]. There is a synergy between the initial injuries of ischemia/reperfusion and acute rejection [37]. Ischemia/reperfusion injury may lead to loss in membrane integrity, the consequences at the organ level being arrhythmias, contractile dysfunction, and loss of adrenergic support [38]. Minimizing reperfusion injury is thus of importance. This is supported by the results showing that recombinant human superoxide dismutase (rhSOD) reduce the number and severity of first acute rejection episodes and improves longtime results in recipients of cadaveric allografts [39]. In xenotransplantation, the issue of ischemia-related graft injury has not really attracted much attention so far. However, we believe that it is likely to be of importance, since all types of xenogeneic immunological mechanisms studied so far have been demonstrated to have a more serious impact than those seen in allogeneic transplantation [40].

Reduction of ROS during ischemia in this concordant non-immunosuppressed xenotransplantation model was not effective in delaying the rejection process under the present experimental conditions. The rejection process in this model, although there is no hyperacute rejection, starts immediately. We know from studies in a model with similar rejection characteristics, the concordant mouse-to-rat model, that the rejection process is rather fierce; even on the first postoperative day, there are histological features of moderate rejection with a mean score of 4.0 on a scale of 0–8 using a system modified from Billingham's criteria, whereas, in the allotransplant setting, this is not seen until day 3–4 [16, 26]. The causes of the falling PCr/β-ATP ratios in the grafts at the time points studied probably have a mixed genesis: the sequel of ischemia-reperfusion and the consequence of the rejection process. Theoretically, there is no reason to believe that the rejection processes per se differed between the groups. This is further supported by our previous report, where mouse grafts transplanted to rats in a similar fashion (an immunologically almost identical model), and followed by *in vivo* $^{31}$P MRS displayed a homogenous histological picture [16]. Furthermore, there was no difference in operation time, technique, temperature, or any other parameters related to the study groups. We therefore believe that the higher PCr/β-ATP ratio in group A vs. control (group B) on day 1 (1.99 vs. 1.43) has to be a consequence of the effect of the induced SOE during the ischemic phase.

The fact that we did not see any effect if SOE was induced post transplantation is not entirely clear. It might be that, in a situation with already ongoing oxidative stress, because of rejection and/or ongoing rep-

erfusion injury, the effect of creating additional ROS in the form of singlet oxygen might be ineffective or further increase the oxidative stress. However, in our rat hind limb ischemia model, where ischemic skeletal muscle was illuminated before, under, and after ischemia, the effect of SOE induction was clearly beneficial for the energetic recovery [6]. It has been proposed that singlet oxygen can be an intermediate in biological processes when present in small concentrations, whereas, in higher concentrations, the effect is detrimental to the cells in the tissue [31, 41]. In our previous *in vitro* study, we could also see a clear and narrow dose range [5]. Thus, the total concentration of singlet oxygen could perhaps become too high if SOE is induced under ongoing reperfusion and rejection in a xenotransplanted heart in contrast to a reperfused skeletal muscle, where there are no immunological incompatibilities involved. The fact that we are dealing with different types of musculature with different sensitivity to ischemia is also an additional factor to be accounted for when comparing the effect of SOE under various conditions.

Photosensitivity to different light sources occurs in biological systems at various levels [5, 31]. There is ample evidence that the mitochondrial respiratory chain components cytochrome c oxidase and NADH dehydrogenase are sensitive to photoexcitation [42–44]. It is also known that photoexcitation may lead to changes in electron transfer rate and in mitochondrial redox activity, which ultimately may modulate ATP production via oxidative phosphorylation [42, 45]. It has been demonstrated that free radicals, possibly singlet oxygen, are generated during illumination with He-Ne lasers at a wavelength similar to the one used here (633 nm vs. 634 nm in our study) [41], and has direct effects on mitochondria resulting in increased ATP synthesis [42]. Similar effects have been reported when illuminating rat hearts before and after cold ischemia with an Argon-dye laser with a wavelength of 660 nm and when studying isolated cardiomyocytes *in vitro* [46]. Thus, one possible effect of the SOE illumination, besides the demonstrated capability to reduce production of ROS [5], might be a stimulatory effect on the respiratory chain increasing the production of HEP like ATP.

We have previously shown that during ischemia and reperfusion, mitochondria can be a source and a target of free radical generation [47, 48]. Supportive evidence has also been provided by Vanden Hoek *et al.*, who demonstrated that the principal sources of the ROS generated during ischemia are the mitochondria [49, 51]. The generated ROS can negatively affect the membrane integrity of mitochondria, leading to impaired oxidative phosphorylation with the end result being less ATP production [51]. The beneficial antioxidative effect of SOE reported *in vitro* in human monocytes was partly attributed to inactivation of NADPH oxidase [5]. The results presented here are more complex. However, we believe that there is reasonable support for the idea that SOE might, at least in part, exert its effect through reduced production of ROS in the mitochondria during ischemia and reperfusion, implicating higher membrane integrity and thus better preservation of ATP. The effect might be mediated through inhibition of mitochondrial NADPH oxidase, thus decreasing the production of mainly superoxide anions. Ongoing studies *in vitro*, as well as *in vivo*, in our laboratory will hopefully shed further light on the mechanisms of action of SOE in situations of ischemia and reperfusion. Of note, preliminary data from our laboratory now indicate an increased survival in SOE-illuminated hamster hearts subjected to moderate to extensive ischemia (8 h of cold ischemia at +4°C) before transplantation (Lukes *et al.*, unpublished observations).

To conclude, we provide for the first time *in vivo* evidence for the capacity of SOE generated by photon illumination at 634 nm to preserve the levels of HEP, measured as the PCr/β-ATP ratio, in a heterotopic concordant hamster-to-rat heart xenotransplantation model. This effect is most likely mediated through the reduction of ROS generated by mitochondria during ischemia leading to mitigated oxidative stress upon reperfusion and thus decreased membrane dysfunction, the effect detected as higher levels of HEP. An alternative explanation might be an increased synthesis of HEP like ATP by a direct effect on the respiratory chain of the mitochondria. This could not, however, delay the rejection process in this model under the present experimental conditions. If reduction of early ischemic events in experimental xenotransplantation, for instance with SOE, and thereby decreased activation of endothelial mechanisms, as is the case in allotransplantation models, has any impact on the future development of chronic rejection features in immunosuppressed xenografts, it remains to be investigated. Preliminary data from our laboratory indicate at least a positive effect on the short-term survival of moderately to severely ischemic hamster hearts in unmodified recipients. More immediate clinical applications of SOE can be foreseen in allotransplantation surgery and in other situations where reperfusion occurs upon previous periods of ischemia. Further studies are clearly indicated to explore the mechanisms of action in greater detail and to investigate the *in vivo* potential of SOE in reducing the consequences of ischemia-reperfusion initiated injurious mechanisms, both per se and as a complement to other treatments.

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