Focusing lung transplant: physiological and antioxidant responses and effect of allopurinol to ischemia-reperfusion oxidative stress under different inspired oxygen concentrations

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Abstract

Background: To evaluate the effect of allopurinol after lung ischemia-reperfusion (IR) injury under ventilation with different inspired oxygen concentrations (0.21 and 1.00 pO₂) on physiological responses and tissue damage and antioxidants present in lung.

Methods: Sixty Wistar rats were randomly allocated to six groups. Group 1 or control group, Groups 2 and 3 animals ventilated during ischemia-reperfusion with 0.21 and 1.00 pO₂, respectively. The three other groups (1A, 2A and 3A) were pre-treated with 100 mg/kg allopurinol and submitted to similar procedures as groups 1, 2 and 3, respectively. The model used was normothermic ischemia-reperfusion in situ. Duration of ischemia and reperfusion was 30 and 10 minutes, respectively. Outcome measures included mean systemic arterial pressure (SAP), partial pressure of oxygen/inspired fraction of oxygen (PaO₂/FiO₂) ratio, lung wet/dry weight ratio, and markers of oxidative stress in lung tissue, such as levels of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH), as well as activity of catalase (CAT) and glutathione peroxidase (GPx).

Results: There was a significant (p<0.05) decrease in the SAP among groups 3x1, 2x2A and 3x3A. With regard to the PaO₂/FiO₂ there was a significant (p<0.05) decrease between groups 3x2 and 3x3A. TBARS levels showed a significant (p<0.05) decrease in group 3 compared to group 3A, while there was a significant (p<0.05) increase in the lung wet/dry weight ratio comparing groups 3x2, 2x 2A and 3x3A.

Conclusions: After IR at normoxia (0.21pO₂) ventilation showed decreased SAP, higher PaO₂/FiO₂ ratio, lower pulmonary edema, and also attenuating lung damage measured as markers of oxidative stress compared to hyperbaric ventilation (1.00 pO₂). In addition, pretreatment with allopurinol was able to improve all these parameters. Ventilation under normoxia and use of allopurinol should be recommended for lung transplantation and organ preservation to attenuate ischemia-reperfusion injury.

Introduction

Relatively high indices of morbidity and mortality usually accompany organ transplantation including lung transplant [1-5]. Ischemia-reperfusion (IR) and its associated injury is a complex phenomenon present in atherosclerosis, trauma, pulmonary embolism, and surgical procedures, such as cardiopulmonary bypass and lung transplant [6]. Reactive oxygen species (ROS) and reactive oxygen species (RNS) overgeneration have been associated with IR injury in different organs [3,4,7-11]. Several characteristics and consequences of IR pathological events are present in different organs and tissues, including lung, however ROS overgeneration and the consequent oxidative stress (OS) seem to be a common feature always present in such cases [12].

Other consequences of IR injury particularly in lungs are also involved, as the onset of an inflammatory process, activation of neutrophils, platelets, cytokines, and the complement system, endothelial cell damage, increased vascular permeability, among several other biochemical and histological processes [3,13,14]. Nevertheless, contrasting with other tissues, lung is the solely organ that cope ischemia avoiding hypoxic conditions due to the proximity of high tensions of oxygen present in the alveoli [5]. In addition, the lung interface, which is characterized by a very delicate structure of the alveolar-capillary membrane, faces a huge damage associated to the deleterious IR process, including the procedure of lung transplant, especially in critically ill patients [15].

Regarding OS in different organs and tissues associated to the IR process, levels of lipoperoxidation and protein carbonyls among other markers of oxidative stress are increased, which are detected after distinct periods of ischemia (minutes or hours) followed by reperfusion [14,16-18]. Also, cytochrome P₄₅₀ seems to be the cytosolic component associated to the increased of mitochondrial hydrogen peroxide concentrations production and the related IR-cell damage.

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In addition, a massive superoxide production is produced during the reperfusion period, thereby favoring a severe OS condition.

The severity of oxidative stress and the consequent damage to important structures and molecules seem to depend either on the time of ischemia [19,20] and/or the time of reperfusion [10,11]. Anyway, ROS overgeneration is strongly associated with reperfusion, i.e., when oxygen resumes to organs and tissues, thereby allowing the restitution of oxidative metabolism, the so-called oxygen paradox or the dual effect of oxygen [14]. In IR singlet oxygen and hydrogen peroxide concentrations and other ROS are increased while main antioxidant enzymes, such as as SOD, CAT and GPX as well as important chain-breaker antioxidants (vitamin E, GSH and others) are decreased [14,20]. Therefore, the inactivation of these important enzymes combined with the lowering of important antioxidants lead to a severe OS condition.

The antioxidant efficacy of allopurinol in protecting different tissues and organs regarding IR-related damage is well documented in the related literature [21-26]. However, as far as we know, no study on the effect of allopurinol was carried out yet considering different ventilation conditions (normoxia and hyperoxia) on several OS markers in lung tissues after IR.

**Material and methods**

Sixty Wistar rats were randomly distributed in six groups (n=10 animals each) as follow: Group 1 was the control group (G1), in group 2 (G2) the animals were ventilated at normoxia (pO2=0.21 atm) during the process of IR (under normothermy – 19-23°C in sita), with ischemia of 10 min of duration followed by reperfusion of 30 min of duration), while in group 3 (G3) the animals were ventilated at hyperoxia (pO2=1.0 atm) during IR. To verify the effect of pretreatment with an antioxidant, allopurinol (A; dose of 100 mg/kg) was administered by gavage 24h (pre-operatory) before the experiments in animals from groups G1A, G2A and G3A.

**Experimental design and surgical procedures**

As physiological parameters, the systemic arterial pressure (SAP), the partial pressure of oxygen and the inspired oxygen fraction (pO2/ FiO2) ratio, were monitored and recorded during the experiment in all six groups. The animals received pre-anesthesia (atropine 0.25 mg/kg), followed by anesthesia. Anesthesia consisted of a mixture of pentobarbital 3% and chloridate of 2-(2,6-xilidino)-5,6-dihydroiso

Determination of thiobarbituric acid reactive substances (TBARS)

Reduced glutathione (GSH) was measured according to Anderson [29], using the Elmann’s reagent (DTNB: 2-dithionitrobenzoic acid). Immediately after the tissue excision, acid extracts were obtained by the addition of the 12% of trichloroacetic acid (1:4 v/v), and vortexed for 30s, and centrifuged at 5,000 g for 10 min. Supernatants from the acid extracts were added with 0.25 mM DTNB in 0.1M Na phosphate, pH 7.4. Homogenizations were carried out on ice (4-5ºC) using a tissue tearor during an interval of 10-15s, followed by centrifugation at 10,000 g for 10 min. The supernatant was used for enzymatic assays. After tissue excision acid extracts were promptly prepared in 12% TCA (1:4, w/v) for the assays of glutathione and thiobarbituric acid-reactive substance (TBARS).

**Antioxidant enzyme assays**

Catalase activity was determined by measuring the decrease of hydrogen peroxide in a 10 mM solution at 240 nm [27]. Glutathione peroxidase was assayed at 340 nm according to Flohé and Gunzler through the glutathione/NADPH/glutathione reductase system, by the dismutation of tert-butyhydroperoxide. All activities were measured in duplicate and expressed in grams of wet lung tissue [28].

**Glutathione assay**

Reduced glutathione (GSH) was measured according to Anderson [29], using the Elmann’s reagent (DTNB: 2-dithionitrobenzoic acid). Immediately after the tissue excision, acid extracts were obtained by the addition of the 12% of trichloroacetic acid (1:4 v/v), and vortexed for 30s, and centrifuged at 5,000 g for 10 min. Supernatants from the acid extracts were added with 0.25 mM DTNB in 0.1M Na phosphate, pH 8.0, and the formation of thiolate anion was immediately determined at 412 nm (ε292 = 14.1 M⁻¹ cm⁻¹). Samples were measured in duplicate and expressed in micromoles per gram of tissue.

**Lipid peroxidation assay**

Determination of thiobarbituric acid reactive substances (TBARS) was used for assessing endogenous lipid oxidation in lung, according to Ohkawa [30] and Bird and Draper [31]. Tissue acid extracts were obtained by the addition of the homogenate to trichloroacetic acid.
12%, vortexed and then centrifuged. Supernatants were centrifuged at 5,000 g for 5 min, added with 0.67% (w/v) 2-thiobarbituric acid, maintained in boiling water for 60 min, cooled at 5°C for 10 min, and then measured spectrophotometrically at 535 nm. Samples were measured in duplicate and expressed in nmol TBARS g tissue⁻¹ using ε₅₃₅ = 153 mM⁻¹ cm⁻¹.

Statistical analyses

Multiple comparison test was performed by INSTAT software (GraphPad, San Diego, CA, USA), and data were analysed by ANOVA for non-parametric values by the Kruskal-Wallis test comparing each group with the others (G1 x G2 x G3), the same in those pretreated with allopurinol (G1A x G2A x G3A). When the initial comparison was significant by Kruskal-Wallis, data were complemented with the multiple comparison test of Mann-Whitney (G1 x G2; G1 x G3; G2 x G3), the same with rats treat or not with allopurinol (G1A x G2A; G1A x G3A; G2A x G3A). Values were expressed as means ± SEM, and p-values less than 0.05 (5%) were considered to be statistically significant.

Results

Physiological parameters

**Systemic arterial pressure (SAP):** No significant differences were obtained for the systemic arterial pressure among the different groups at each time zero (time 0), when compared to each other using the Kruskal-Wallis test (p=0.3704). However, when comparing all the experimental period (0→40 minutes, i.e., after considering all the IR experimental periods together), a significant difference was detected (p=0.0368*; Kruskal-Wallis test; Table 1). Using the complementary Mann-Whitney test, animals submitted to hyperoxia (G3=100% FiO₂) showed a decreased (p=0.0154*) systemic arterial pressure compared to controls (Sham), while no differences were detected for G2 x G3 (p=0.3845) and G1 x G2 (p=0.0751)(Table 1). Pretreatment with allopurinol (A) was able to decrease the systemic arterial pressure in all comparisons, i.e., normoxia conditions compared either to controls (G1 x G2A; p=0.0162*), inside the normoxia group (G2 x G2A; p=0.0033*), as well as inside the hyperoxia group (G3 x G3A; p=0.0006***), considering the period of time 0→40 minutes.

**pO₂/FiO₂ ratio:** The statistical analysis for the pO₂/FiO₂ ratio under different ventilation conditions showed no differences among the groups at time zero (p=0.5792; Kruskal-Wallis test). However, when all the experimental period (040 minutes) is considered a very significant difference was obtained (p= 0.0001*; Kruskal-Wallis test). Using the complementary Mann-Whitney test for the comparison among the three groups, and similar to the results found for the systemic arterial pressure, only the group submitted to hyperoxia ventilation showed a decreased ratio compared to rats ventilated under normoxia ventilation (p=0.0002***). as well as compared to controls (p=0.0002***), while

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**Figure 1.** Flow sheet for cases.
the comparison between normoxia versus controls was not significant (p=0.9397; Table 1).

When taken into consideration the effect of allopurinol pretreatment under different ventilation conditions again no significant differences among the groups at time zero were detected (p=0.8824; Kruskal-Wallis test). On the other hand, when considering all the experimental period (time0 to 40 minutes) all comparisons showed significant decreases of the pO2/FiO2 ratio, either at normoxic ventilation compared to controls (G1xG2A; p=0.0162*), inside the normoxia group (G2xG2A; p=0.0033*), as well as inside the hyperoxia group (G3xG3A; p=0.0006**)(Mann-Whitney test; Table 1).


**Table 1.** Systemic arterial pressure (SAP, in mmHg) and (FiO2) in different experimental times (t0, t30, and t40) of controls (G1), lungs ventilated at normoxia (G2) or hyperoxia (G3), "A" after each group at the lower part of the table means animals pretreated with Allopurinol.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP(t0)</td>
<td>109.0±8.0</td>
<td>117.8±3.4</td>
<td>117.7±7.3</td>
</tr>
<tr>
<td>SAP(t30)</td>
<td>112.5±11.3</td>
<td>104.0±13.1</td>
<td>98.3±15.1</td>
</tr>
<tr>
<td>SAP(t40)</td>
<td>106.1±12.8</td>
<td>-2.9±11.0</td>
<td>-11.7±10.8</td>
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<tr>
<td>ΔSAP</td>
<td>2.97±34.4</td>
<td>24.4±18.1</td>
<td>-29.0±15.0***</td>
</tr>
<tr>
<td>ΔpO2</td>
<td>553.6±159.5</td>
<td>579.0±90.4</td>
<td>528.2±113.0</td>
</tr>
<tr>
<td>FiO2(t0)</td>
<td>682.7±141.6</td>
<td>736.9±97.2</td>
<td>347.9±54.6</td>
</tr>
<tr>
<td>FiO2(t30)</td>
<td>689.4±145.6</td>
<td>712.1±97.8</td>
<td>363.5±51.1</td>
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<tr>
<td>Parameters</td>
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<td>G2A</td>
<td>G3A</td>
</tr>
<tr>
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<td>110.5±8.0</td>
<td>107.0±12.5</td>
</tr>
<tr>
<td>SAP(t30)</td>
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<td>104.5±12.1</td>
<td>103.5±10.3</td>
</tr>
<tr>
<td>SAP(t40)</td>
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<td>104.5±8.6</td>
<td>104.5±10.4</td>
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<td>ΔSAP</td>
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<td>-5.4±6.6**</td>
<td>-1.8±8.8***</td>
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<td>21.3±30.7</td>
<td>12.6±7.8***</td>
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<tr>
<td>FiO2(t0)</td>
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<td>553.5±155.2</td>
<td>548.7±46.8</td>
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<tr>
<td>FiO2(t30)</td>
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<td>640.4±120.4</td>
<td>504.0±42.5</td>
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<tr>
<td>FiO2(t40)</td>
<td>650.4±104.4</td>
<td>635.2±81.9</td>
<td>476.6±24.0</td>
</tr>
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</table>

Table 2. Biomarkers of oxidative stress in lung tissue of controls (G1), lungs ventilated at normoxia (G2) or hyperoxia (G3). A after each group at the lower part of the table means animals pretreated with Allopurinol. Activity of CAT (catalse; mmol min⁻¹ g wet tissue⁻¹), GPx (Glutathione peroxidase; mmol min⁻¹ g tissue⁻¹), contents of GSH (reduced glutathione; mmol g⁻¹) and TBARS (lipoperoxidation; nmol TBARS g⁻¹).

<table>
<thead>
<tr>
<th>Markers</th>
<th>Controls (G1)</th>
<th>Normoxia (G2)</th>
<th>Hyperoxia (G3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT</td>
<td>0.94±0.34</td>
<td>0.10±0.38</td>
<td>0.14±0.40</td>
</tr>
<tr>
<td>GPx</td>
<td>453.4±105.7</td>
<td>343.2±54.56</td>
<td>512.1±161.4</td>
</tr>
<tr>
<td>GSH</td>
<td>0.97±0.38</td>
<td>1.13±0.31</td>
<td>1.03±0.28*</td>
</tr>
<tr>
<td>TBARS</td>
<td>50.2±38.13</td>
<td>40.3±39.33</td>
<td>59.32±38.34</td>
</tr>
</tbody>
</table>

Different superscript letters denote significant differences applying Kruskal-Wallis test followed by the complementary Mann-Whitney test according to: (*) G1A x G1 (controls); (α) between G1xG2; (β) between G1xG3; (γ) between G2xG2A; (δ) between G2xG3A; (ε) between G3xG3A; (ξ) between G1AxG3A. Detailed and specific values for each p (p<0.05, p<0.01, or p<0.001) are in the Result section.

**Marker of OS – Lipoperoxidation levels (TBARS):** In the present study TBARS contents in lung tissue showed only a tendency to increase under ventilation not protected with allopurinol, however this tendency showed a relationship with the higher FiO2. The values found detected when compared the different groups to each other. However, when considering the entire experimental period (0-40 minutes) corresponding to the IR experimental period, a significant difference was detected for the ischemic lung (p=0.0368; Kruskal-Wallis test; Table 1). Using the complementary Mann-Whitney test, animals submitted to hyperoxia (G3=100% FiO2) showed a decreased ratio compared to controls (G1 vs. G3; p=0.0017***) or to normoxia exposed lungs (G2 vs. G3; p=0.0010***) were detected (Table 1). On the other hand, pretreatment with allopurinol (A) compared to rats not pretreated with the antioxidant, was able to decrease the ratio in all comparisons, showing very significant differences. More specifically, comparing the effect of allopurinol inside the control group (G1xG1A; p=0.0003***), inside the normoxia group (G2xG2A; p=0.0015**), as well as inside the hyperoxia group (G3xG3A; p=0.0002***), in all of them the antioxidant treatment was very effective in decreasing the damage insult in the ischemic lungs.
after the Kruskal-Wallis test detected not significant values between the different groups, either for the left ischemic lung (p=0.2117) nor the right lung (p=0.1852). Considering the effect of allopurinol comparing both lungs under different ventilation conditions, values for the left lung (p = 0.1814) and right lung (p = 0.7783) showed a similar absence of statistical significance among the three groups, even after applying the complementary Mann-Whitney test (Table 2). However, when the effect of allopurinol was compared with animals not previously treated with this antioxidant after applying the Mann-Whitney test significant differences were detected, either for the ischemic lung exposed to hyperoxia (G2 vs. G3A; p=0.0188*), as well for the right lung in animals of the control group (G1 vs. G1A; p=0.0306*)(Table 2). The other comparisons were not significant for both lungs, the left lung (G1 vs. G1A; p=0.6498; G2 vs. G2A; p=0.9397) and the right lung (G2 vs. G2A; p=0.5960; G3 vs. G3A; p=0.6217) (Table 2).

Antioxidants – GSH: The effect of ventilation at different oxygen tensions and also considering the effect of allopurinol in both lungs on the responses of GSH revealed significant differences only for the left ischemic lung using the Kruskal-Wallis test (p=0.3362; Table 2). Also, using the Kruskal-Wallis test when comparing to controls, GSH contents in the ischemic left lung submitted to normoxic ventilation (G1) also showed a significant difference (p=0.0062*), as well as under hyperoxia (p=0.0375*; Table 2). Significant differences were also detected applying the complementary Mann-Whitney test between different ventilations in the left lungs ventilated under hyperoxia and normoxia (Table 2), excepting the comparison between G1 vs. G3 (p=0.8798), which was not significant. Thus, the difference between G2 vs. G3 showed a very significant value (p=0.0099***), while that obtained for G1 vs. G3 was less significant (p=0.0375*).

On the other hand, when considering all the effect of allopurinol, Kruskal-Wallis test detected a significant difference for the ischemic lung (p=0.0505*), but not for the right lung (p=0.6921). The statistical analysis inside each group revealed significant differences for ventilation at normoxia compared to controls (G1xG2A; p=0.0494*) and ventilation at hyperoxia compared to controls (G1xG3A; p=0.0494*), while no significant difference appeared between the effect of allopurinol comparing normoxia and hyperoxia ventilation (G2xG3A; p=0.7620; Mann-Whitney test; Table 2).

Antioxidant Enzymes (GPx): Using the Kruskal-Wallis test for glutathione peroxidase activity no significant differences were detected either for the ischemic lung (p=0.9658), as well for the right lung (p=0.0748), for animals not pretreated with allopurinol (Table 2). However, pretreatment with allopurinol gave significances when comparing both lungs each other or with controls by the Kruskal-Wallis test, for the left ischemic lung (p=0.0053*), as well as for the right lung (p=0.0408*). After applying the Mann-Whitney test (Table 2) pretreatment with allopurinol (A), the ischemic lung showed significant difference comparing G1A vs. G2A (p=0.0343*), G2A vs. G3A (p=0.0413*), while the left lung ventilated under hyperoxia and compared to controls revealed no significant difference (p=0.9698). For the non-ischemic right lung no significant statistical results were obtained for the two ventilations compared to controls (G1A vs. G2A; p=0.1124 and G1A vs. G3A; p=0.3445), while the comparison for normoxia versus hyperoxia ventilation revealed a significant difference (G2A vs. G3A, p=0.0140*). Furthermore, when the mean values of rats ventilated with and without pretreatment with allopurinol were compared, significant differences were revealed in all three groups for both lungs by the Mann-Whitney test (Table 2). For the ischemic lung the following results were obtained: G1 vs. G2A (p=0.0156*), G2 vs. G2A (p=0.0005**), G3 vs. G3A (0.0007***). For the non-ischemic lung: G1 vs. G2A (p=0.0233*), G2 vs. G2A (p=0.0033***), G3 vs. G3A (0.0222***)

Antioxidant Enzymes (CAT): Surprisingly and contrary to the other results obtained regarding oxidative stress markers, glutathione peroxidase activity showed no significant differences in the ischemic lung (p=0.0800), while for the right lung (p=0.0396*), a significant difference was detected using the Kruskal-Wallis test, while no difference appeared for the right lung (p=0.3064; Table 2). The complementary Mann-Whitney test revealed a significant difference (p=0.0284*) comparing CAT values for the right lung (G2 vs. G3; Table 2). The pretreatment with allopurinol showed significances only for the ischemic left lung (p=0.0399*) when comparing both lungs through the Kruskal-Wallis test (Table 2). Similar to the response obtained for GPx, after applying the Mann-Whitney test, a very significant difference (p=0.0101*) was detected for the left lung, comparisons inside each group under distinct ventilation conditions in animals with and without allopurinol (G2A vs. G3A; Table 2). On the other hand, for when comparing animals treated and non-treated with allopurinol, the right lung showed no significant differences between controls G1xG1A (p=0.0695), while the comparison between G2xG2A (p=0.0052*) and G3xG3A (p=0.0065*), such comparison were very significant (Table 2). The left ischemic lung showed significant differences in all three groups by the Mann-Whitney test (Table 2), when comparing G1 vs. G1A (p=0.0041*), G2 vs. G2A (p=0.0082*), and G3 vs. G3A (p=0.0003*) (Table 2).

Discussion

Physiological parameters

Pretreatment with allopurinol decreased the systemic arterial pressure while preserving the pO_2/F_iO_2 ratio comparing to controls (Table 1), demonstrating its antioxidant ability. Similar finding was obtained by Zhao and collaborators [19] in rat lung. Allopurinol is effective in decreasing the edema and OS related to IR [19,25]. Furthermore, the ratio wet/dry lung weight reflects the secondary edema to IR injury in different studies, and the very significant difference obtained for this parameter evaluated in the present study (0.0032*) is in accordance to other similar studies. Furthermore, we have also measured the pO_i/F_iO_2 ratio, which is considered an indirect evaluation of the endothelium function. Lung edema is related to higher capillary permeability due to endothelium disfunction, which in turn is related to ATP decrease, as well as to the release of several inflammatory mediators and also to high ROS overproduction [32]. Accordingly, in our study the group submitted to hyperoxia showed a decreased value for this ratio, thus inferring the damage related to capillary permeability.

Considering that only the animals submitted to hyperoxia (G3=100% F_iO_2) showed a decrease in the systemic arterial pressure when compared to controls (Sham operation) as well as those exposed to normoxia, probably indicating that the lung tissue was damaged by ROS overgeneration when high partial pressures of oxygen are present. This result is in accord to Haniuda and collaborators [33], who also detected enhanced lipoperoxidation levels in lungs preserved at moderate hypothermia. In addition and similar to the results found for the systemic arterial pressure, the pO_i/F_iO_2 ratio showed a decreased ratio compared to normoxia (G2) and the control group (G1). This suggest that under high oxygen concentration the alveolar/capillary membrane integrity was probably jeopardized [34], which is in accord to other related studies [35,36]. It is also important to stress out that...
lungs function after transplant can be also improved by a relatively slow increase in both ventilation rate, as well as in blood flow during reperfusion [37].

Marker of OS – Lipoperoxidation levels (TBARS)

In the present study TBARS contents in lung tissue showed only a tendency to increase under ventilation not protected with allopurinol, however this tendency showed a relationship with the higher FiO2. This response is similar to those found in related studies involving lung IR injury [38,39] the former authors also described a similar damage to proteins through protein carbonyl levels. Accordingly, when ventilation of lungs not pretreated with allopurinol was compared, higher TBARS levels were detected in the hyperoxia group, in other words, the previous treatment with this antioxidant was able to decrease OS damage in the ischemic lung. Fisher and collaborators [8] also showed a protective effect of allopurinol decreasing lipoperoxidation during IR in rats. Furthermore, such protection against lipid damage was also demonstrated in rats through a preservation solution containing allopurinol [40].

Allopurinol is analogous to hypoxanthine, thereby acting as an xanthine oxidase inhibitor and leading to a lower generation of superoxide anion [14]. In this sense oxipurinol, the major metabolite derived from allopurinol, is a very efficient scavenger of the deleterious, hydroxyl radical (•OH) [41], which is the main ROS responsible for the damaging cellular and organelle membranes. Lipoperoxidation is the oxidation of unsaturated lipids and cholesterol in membranes as well as in lipoproteins, which is promoted by ROS. This lipid oxidation process is considered the most deleterious consequence of IR in different organs and tissues [42,43].

It is worth to stress out that measurements of lipoperoxidatin as TBARS, as well as other markers of oxidative insult, are often used for the evaluation of tissue lesion associated to IR injury [44-46]. Also, in spectrophotometric TBARS assay, malondialdehyde (MDA) is the major (more than 90%) among other toxic dialdehydes produced by lipid oxidation especially on cell membranes [14].

Antioxidants – GSH

Compared to controls, a progressive depletion of GSH contents in the ischemic lung tissue was revealed, both under normoxia and hyperoxia (21% and 100% FiO2, respectively), clearly indicating that hyperbaric ventilation jeopardized the glutathione protection. GSH is a widespread endogenous and generalist antioxidant (Wilhelm Filho et al., 2002), being very effective in quenching of ROS and RNs. In this sense, ‘NO is toxic only in the early stages of reperfusion because of its interaction with superoxide anion (O₂–’), thus forming the very reactive and deleterious peroxinitrate (ONOO–).

In the present study a significant difference in GSH concentrations in tissue lung was also obtained between hyperoxia and normoxia ventilations. Such response is probably reflecting the higher ROS generation related to hyperoxia ventilation, GSH being depleted to compensate and attenuating OS in lung tissue. Depletion of reduced glutathione concentrations in severe OS conditions is critical for cell homestasis. Such OS condition and GSH depletion were present in patients with bronchiolitis obliterans syndrome (BOS), which is considered is the most serious long-term sequel of lung or heart-lung transplantation.

As a matter of fact, OS and GSH depletion is always detected in patients undergoing organ transplantation [47]. GSH levels being rapidly decreased while producing high levels of its oxidized form, which is very toxic to cells and tissues (GSSG).

GSH present in a preservation fluid prevents pulmonary edema following lung transplantation in dogs. The pretreatment with allopurinol was also able to prevent GSH depletion associated with hyperoxia ventilation (this study).

Antioxidant enzymes

No significant differences were detected for the two antioxidant enzymes evaluated in rats submitted to IR and not pretreated with allopurinol regarding the three groups examined. The same result was described by Bryan and collaborator [45]. On the other hand, when ventilation of lungs not pretreated with allopurinol was compared to rats protected with the pretreatment of allopurinol, both enzymes decreased their activity, probably accompanying the decrease of hydrogen peroxide concentrations promoted by allopurinol. GPx and CAT are the two main antioxidant enzymes present in the intracellular environment responsible for hydrogen peroxide detoxification into water and oxygen. Thus, ROS especially considering hydrogen peroxide and other peroxides, such as those generated through the lipoperoxidation processes produced by IR, induced GPx and CAT activity to compensate the associated OS [48]. It is well known that higher cell concentrations of hydrogen peroxide produces the radical hydroxyl, via the Fenton reaction catalyzed by transition metals, or via the Haber-Weiss reaction, when combined with superoxide anion. In this regard, significant iron-generated OS was already demonstrated in lung transplantation, which might be exacerbated by nitric oxide (‘NO), particularly in BOS [49].

Also, as previously mentioned, the antioxidant allopurinol is able to decrease OS damage in the ischemic lung, thereby decreasing the ratio wet/dry lung weight, which reflects the lung edema [25,19]. Furthermore, in the present study the pO₂/FiO₂ ratio was decreased in rat lungs submitted to hyperoxia, similar to the results described in other related studies [32]. In summary, all the physiological responses seem to be closely associated with the antioxidant responses, acting in a concerted manner to compensate tissue and cellular damage following lung IR process.

Accordingly, the pretreatment with vitamin C was also able to attenuate lung injury after ischemia(3h)-reperfusion(1h) in the heart of rats, which also showed decreased plasma leukocyte sequestration [50]. Also, in vivo experiments in pigs showed that chemiluminescence measurements in animals previously supplementation with vitamins C and E were able to attenuate injury associated to ischemia followed by reperfusion [2], as well as CAT immunotargeting to the pulmonary endothelium also decreases IR [51]. Several other antioxidant supplementation studies were also effective to demonstrate the beneficial effect of such interventions in lowering OS associated with IR in different organs and tissues [14].

Nitric oxide (‘NO) also plays an important role in OS related to IR in different organs. In this regard, ‘NO seems to be toxic only in the early stages of reperfusion because of its interaction with superoxide anion (O₂–’), thus forming the peroxinitrate (ONOO–), which is not a radical species but a very deleterious and diffusible RNS. Interestingly and corroborating the general results obtained in the present study under ventilation at 0.21 atm FiO2 only at normoxic condition the inhalation of ‘NO during lung ventilation was able to promote a significant and pronounced decrease in the mean pulmonary pressure in transplanted lungs in pigs.
At last and not least, it is interesting to realize that IR process is a physiological and normal routine sustained by some animals in nature, especially diving mammals, thus enabling them to overcome the systemic oxidative stress related to IR [52]. These organisms possess a constitutive and relatively high antioxidant system in blood and in different organs to continuously compensate the IR insult associated with alternated surface breathing and long apnea-related ischemic periods of diving compared to non-diving mammals.

Conclusions

The ischemic-reperfusion process in lungs using ventilation at normoxia (0.21pO₂) compared to hyperbaric ventilation (1.00 pO₂) showed decreased systemic arterial pressure (SAP), higher PaO₂/FiO₂ ratio, higher wet/dry weight ratio in the ischemic lung, and lower pulmonary edema, as well as attenuating lung damage associated to several markers of oxidative stress here examined. On the other hand, pretreatment with allopurinol was able to improve all these parameters. Ventilation under normoxia and use of allopurinol should be recommended for lung transplantation and organ preservation to attenuate ischemia-reperfusion injury.

References

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