Mannitol Prevents Acute Lung Injury After Pancreas Ischemia-Reperfusion: A Dose-Response, Ex Vivo Study

Avi A. Weinbroum

Abstract Oxidants and their generator, xanthine oxidase (XO), play a major role in the damaging of the structural and functional integrity of the lung. Such damage has been recently demonstrated in the presence of pancreas ischemia–reperfusion (IR). We investigated whether mannitol, a clinically used agent and antioxidant, prevented lung damage after pancreas IR. Rats (n = 48) were anesthetized, after which each pancreas was isolated and perfused (controls), or made ischemic (IR) for 40 min, or made ischemic and treated upon reperfusion with four different doses of mannitol administered in the perfusate (8 replicates/group). Ischemia was followed by in-series 15-min pancreas plus normal isolated lung reperfusion. Isolated lungs were subsequently perfused for 45 min with the 15-min accumulated effluents. Pancreas injury occurred in all IR organs as demonstrated by abnormal reperfusion pressure, the wet-to-dry ratio, amylase and lipase leakage into the circulation, and XO activity and reduced glutathione (GSH) pool in the tissues. Pulmonary plateau pressure increased by 80%, and final $P_{O_2}/Fi_{O_2}$ decreased by 28% in the IR-untreated paired lungs. Bronchoalveolar lavage volume increased by 50% and 2- to 8-fold increase in their contained XO and GSH were recorded as well. The above indices of injury in lungs perfused with 0.77 mM mannitol were the least detected, compared with negligible efficacy of other (0.55 < 0.22 < 1.1 mM) dosages. Amylase and lipase did not contribute to lung injury. Ex vivo acute pancreatitis induces acute lung injury via oxidants/antioxidants imbalance, which is preventable by mannitol.

Keywords Pancreas · Ischemia-reperfusion · Lung Injury · Oxidants · Mannitol

Introduction

Warm ischemia–reperfusion (IR) of the pancreas is associated with microcirculatory derangements (e.g., increased vascular permeability, arterial constriction, stasis of capillary perfusion) and pancreatic enzyme elevation [1, 2]. The systemic consequences of acute pancreatitis resemble those reported after hepatic or intestinal IR [3, 4]. We had previously documented the participation of oxidants in the evolution of ALI [3, 4, 6–9]. Xanthine oxidase (XO) emerged as a significant, if not the primary, source of stress oxidants: it increased hundreds of times after various stress events, both in animals and humans [3, 4, 6–10]. The pivotal role of XO in inducing lung secondary organ injury was confirmed in pancreas IR as in several other studies, using XO oxidoreductase inhibitors (e.g., allopurinol, sodium tungsten) [6–11].

The purpose of this study was to observe whether pancreas IR-induced ALI could be attenuated or even prevented by mannitol—a clinically used and antioxidant agent. We used four different doses of mannitol in the attempt to reduce XO activity in a rat ex vivo pancreas-lung dose-response model.

Materials and Methods

This study was performed in accordance with the Public Health Service policy on Humane Care and Use of Laboratory Animals, the National Institute of Health (NIH)
Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act, and was approved by the Institutional Animal Care and Use Committee of Tel-Aviv Sourasky Medical Center.

Organ Preparation and the Double-Organ System

Adult male Wistar rats (\( n = 96 \)), weighing 350–420 g, were anesthetized with intraperitoneal ketamine and diazepam (50- and 5 mg kg\(^{-1}\), respectively). All experiments were performed in an environmental chamber designed to control temperature and minimize water evaporation. One-half of the animals donated pancreases and the others donated lungs. After laparotomy, the pancreases were isolated and perfused according to the method described by Fujimoto et al. [12]. Another group of animals underwent tracheotomy and their lungs were ventilated with 95% air–5% CO\(_2\) with a piston-type rodent ventilator (10 ml kg\(^{-1}\) tidal volume at a rate of 40 breaths min\(^{-1}\)), after which a thoracotomy was performed. The lungs were isolated and perfused as reported elsewhere [3, 6–8] with hemoglobin-free, modified, 5% (weight volume\(^{-1}\)) bovine serum albumin (BSA)-enriched Krebs-Henseleit (Krebs) solution. The lungs were then suspended from a force displacement transducer (Grass Instruments Co., Quincy, MA, USA). The double-organ perfusion system used in this study has been described in detail elsewhere [3, 6]. Two separate peristaltic pumps were used to perfuse the pancreas and the paired lungs. Pre- and post-organ perfusate always passed through an in-line warmer and membrane oxygenator as well as thermometers. Ventilatory and perfusion pressures (see below) were continuously recorded via a hemodynamic monitor (CS/3\textsuperscript{TM}, Datex-Ohmeda\textsuperscript{®}, Helsinki, Finland).

The pancreases were always perfused with Krebs in a single-pass mode, whereas the lungs were perfused in a single-pass (during the conjoint phase) or in a closed-loop recirculation mode (during stabilization and after the conjoint perfusion, see below).

Drug and Experimental Protocol

Mannitol possesses hydroxyl radical scavenging properties both in the circulation and within the organs. We used mannitol in various doses to study its effect in attenuating the induction of pancreatitis-induced ALI. The dosages had been analyzed and used for similar purposes in an earlier study [13]. Mannitol (Baxter, Ashdod, Israel) was added to the Krebs + albumin solution and administered to the pancreases of the designated groups, starting at the 15-min reperfusion phase, with the intent of counteracting the production of reactive oxygen species (ROS) as would be done in the clinical setting [14]. The osmolarity of these solutions ranged from 302–308 mOsm. Several previous studies had excluded any effects of mannitol or albumin upon laboratory analyses [3, 15–17].

The pancreases were divided into six groups, according to whether they underwent ischemia and according to the dose of mannitol with which the IR organs were reperfused immediately after terminating the ischemia. The paired lungs were never subjected to ischemia. One group (\( n = 8 \) pairs/group) served as nonischemic controls (control), and the other five groups consisted of pancreases that had been subjected to ischemia (IR-0) or IR pancreases that had been reperfused with 0.22, 0.55, 0.77, or 1.1 mM mannitol in the Krebs solution (IR-0.22, IR-0.55, IR-0.77, IR-1.1, respectively).

After 30 min of pancreas stabilization (Fig. 1), all IR pancreases were rendered ischemic by stopping the flow for 40 min as previously described [9]; the control organs were continually perfused. In the meantime, the lungs were isolated and stabilized so that the effluent was shuttled from the pancreas into the pulmonary circulation at the end of the 40-min ischemia period. After 15 min of in-series pancreas + lung reperfusion (with or without mannitol), the pancreas was removed from the circuit and the accumulated effluent was circulated through the isolated lung in a closed-loop manner for another 45 min. A 15-min reperfusion of the pancreas allowed the induction of local and remote organ damage by the released compounds [3, 9, 18]. During the 45 min of lung-reperfusion, there is an observable gradual building up of lung damage. Finally, since the flow rate of the pancreas is two to three times lower than that of the lung, an additional pump simultaneously and in a synchronized manner pumped fresh Krebs (the missing volume between the lung and the pancreas flow rates) into the pulmonary circulation during the 15-min in-series period.

Determination of Organ Parameters

Pancreas viability vs. damage was assessed following the protocol of our previous isolated-perfused double-organ model, i.e., by changes in the entering perfusion pressure, the exiting perfusate content and the wet-weight-to-dry weight ratio (WDR) [9]. Pulmonary perfusion pressure, plateau ventilatory pressure, and changes in lung weight during perfusion were measured and continuously recorded. Lung \( P_{O_2}/FIO_2 \) was calculated later. We had previously demonstrated that pulmonary capillary pressure and airway compliance are associated with pulmonary edema closely and directly correlate with perfusion pressure and ventilatory plateau pressure, respectively [3, 9]. As such, we omitted the former in the present report.

At the end of each experiment, the airways were gently flushed three times with 1 ml of warm saline through the trachea, and the fluid was gently sucked out. Markers of
altered bronchoalveolar lavage (BAL) (e.g., increased regained volume and/or content) indicated the presence of abnormal alveolocapillary permeability [3].

Biochemical Analyses

Aliquots of 1.0 ml of effluent were collected for laboratory analyses every 15 min throughout the experiment, with additional time points at 2, 5, and 10 min during the in-series reperfusion phase. Samples were processed in duplicate within 24 hours from the experiment, and tissues were assayed as well. Amylase and lipase concentrations in the pancreas-exiting perfusate indicate pancreatic damage [19, 20]. They were determined by using standard methods and kits for automated analyses (Roche–Boehringer Mannheim GmbH Diagnostics, Mannheim, Germany and Hitachi 747 Analyzer, Tokyo, Japan). The total activity of XO plus its reduced form, XDH, also was assessed (following Hashimoto’s method with modifications [21]). Activity was quantified spectrophotometrically by monitoring the formation of uric acid from xanthine at 292 nm. One unit of activity was defined as 1 μmol min⁻¹ of uric
acid formed at 37°C, pH 7.5. Activity was expressed in mU ml⁻¹ for fluids and mU g⁻¹ wet-weight for tissues.

Reduced glutathione (GSH) is an intracellular low molecular weight thiol that exerts protective activities, primarily intracellularly [22]. In the presence of glutathione deficiency, brain mitochondria may be damaged due to the accumulation of hydrogen peroxide and the lack of the protective glutathione activity [23]. GSH was analyzed in fluids and in fresh organ specimens (Calbiochem #354102 kit, San Diego, CA, USA) and expressed as mM and µmol g⁻¹ dry weight tissue, respectively.

All organs were weighed at the completion of the experiments. Portions were maintained in an oven at 70°C for 5 days and then reweighed to calculate the WDR.

Statistical Analyses

The data variables are summarized as means ± standard deviations. A post hoc analysis was performed at each time point by the analysis of variance (ANOVA) with comparisons between group means using the Student-Newman-Keuls’ test. Trends in each group were compared by ANOVA with repeated measures. The significance level was set at \( P < 0.05 \).

Results

Pancreas Data

During stabilization, pancreas perfusion pressure was similar in all groups (41–48 mmHg) and remained unchanged in the control group throughout their experiments. The 40-min IR produced edema, as expressed by the increase in pancreas WDR (8–92%) in the IR organs compared with their corresponding controls (Table 1), and by the perfusion pressure, which increased by ~50% within 2–3 min of reperfusion.

The biochemical profiles of the various pancreatic effluents are displayed in Table 2. Amylase levels increased ~twofold in all IR groups compared with controls, starting at 2 min into reperfusion and remaining elevated thereafter. Lipase also increased in the reperfusates to values as high as fivefold those recorded during stabilization. The total XO activity in the pancreatic exiting perfusates increased multifold in all IR groups but less in the IR-0.77 group. The GSH was higher in all IR groups’ perfusates than that of the controls, suggesting cytolyses and a high activation rate due to the oxidative insult. By the end of the 15-min reperfusion, most compounds were detected lower compared with the 2-min peaks.

The total XO activity and GSH content in the pancreas tissues that had been determined at the end of the experiments are displayed in Fig. 2. XO activity in the IR-0 pancreas was ~10- to 12-fold lower than that in the controls and in the IR-0.77 organs, and ~2- to 8-fold lower than the values for the rest of the pancreases. Taken together with the circulating data, these results are indicative of tissue lacking the augmented oxidative process due to cytolyses. GSH concentration in the control group was ~fourfold higher than the concentration in the IR-0 organs and 2–3 times higher than the concentrations in the IR-treated groups, representing an augmented GSH pool but eventually consumed following the pancreas oxidant/antioxidant imbalance.

Lungs Data

Pulmonary perfusion pressure and ventilatory plateau pressure were similar for all the lungs during the stabilization period (Fig. 3, lower and upper planes, respectively). During reperfusion, they did not change significantly in the controls or IR-0.77 groups. Each parameter increased threefold during reperfusion and by 50% above stabilization values in the lowest and highest dose regimen groups, respectively. The values of the IR-0.55 group fell between those of the other groups.

All the lungs remained isogravimetric during stabilization, and the control and the IR-0.77 lungs did not gain

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Organs wet-weight to dry-weight ratio (WDR) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Pancreas</td>
</tr>
<tr>
<td>Control</td>
<td>1.3 ± 0.4⁴</td>
</tr>
<tr>
<td>Ischemia-reperfused, untreated (IR-0)</td>
<td>2.5 ± 0.8*</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.22-treated (IR-0.22)</td>
<td>2.1 ± 0.7*</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.55-treated (IR-0.55)</td>
<td>1.9 ± 0.5*⁴</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.77-treated (IR-0.77)</td>
<td>1.4 ± 0.6⁴</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-1.1-treated (IR-1.1)</td>
<td>3.1 ± 0.5*</td>
</tr>
</tbody>
</table>

* \( P < 0.01 \) vs. the corresponding control or the IR-0.55 groups; † \( P < 0.01 \) compared with the IR-0 group

IR ischemia-reperfused organs
### Table 2 Contents of pancreas effluents (mean ± SD)

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Amylase (U l⁻¹)</th>
<th>Lipase (U l⁻¹)</th>
<th>Total XO (mU ml⁻¹)</th>
<th>GSH (mM l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>365 ± 47</td>
<td>263 ± 51</td>
<td>0.137 ± 0.007</td>
<td>0.029 ± 0.003</td>
</tr>
<tr>
<td>Ischemia-reperfused, untreated (IR-0)</td>
<td>341 ± 32</td>
<td>259 ± 47</td>
<td>0.121 ± 0.011</td>
<td>0.031 ± 0.003</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.22-treated (IR-0.22)</td>
<td>350 ± 37</td>
<td>265 ± 46</td>
<td>0.140 ± 0.010</td>
<td>0.025 ± 0.002</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.55-treated (IR-0.55)</td>
<td>345 ± 44</td>
<td>271 ± 30</td>
<td>0.133 ± 0.007</td>
<td>0.024 ± 0.014</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.77-treated (IR-0.77)</td>
<td>349 ± 51</td>
<td>267 ± 33</td>
<td>0.130 ± 0.008</td>
<td>0.032 ± 0.005</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-1.1-treated (IR-1.1)</td>
<td>354 ± 60</td>
<td>270 ± 37</td>
<td>0.130 ± 0.007</td>
<td>0.032 ± 0.004</td>
</tr>
</tbody>
</table>

**Stabilization 30 min**

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Amylase (U l⁻¹)</th>
<th>Lipase (U l⁻¹)</th>
<th>Total XO (mU ml⁻¹)</th>
<th>GSH (mM l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>404 ± 63</td>
<td>268 ± 67</td>
<td>0.175 ± 0.09</td>
<td>0.024 ± 0.01</td>
</tr>
<tr>
<td>Ischemia-reperfused, untreated (IR-0)</td>
<td>884 ± 71*</td>
<td>1441 ± 153*</td>
<td>0.52 ± 0.08*</td>
<td>0.045 ± 0.008*</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.22-treated (IR-0.22)</td>
<td>719 ± 67*</td>
<td>1263 ± 131*†</td>
<td>0.46 ± 0.06*</td>
<td>0.035 ± 0.005*</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.55-treated (IR-0.55)</td>
<td>567 ± 49*†</td>
<td>1021 ± 119*†</td>
<td>0.26 ± 0.07*†</td>
<td>0.035 ± 0.009*</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.77-treated (IR-0.77)</td>
<td>398 ± 77†</td>
<td>288 ± 68†</td>
<td>0.175 ± 0.04†</td>
<td>0.030 ± 0.002†</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-1.1-treated (IR-1.1)</td>
<td>538 ± 56*†</td>
<td>588 ± 91*†</td>
<td>0.35 ± 0.07*†</td>
<td>0.045 ± 0.018</td>
</tr>
</tbody>
</table>

**Reperfusion 2 min**

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Amylase (U l⁻¹)</th>
<th>Lipase (U l⁻¹)</th>
<th>Total XO (mU ml⁻¹)</th>
<th>GSH (mM l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>412 ± 16</td>
<td>255 ± 49</td>
<td>0.196 ± 0.05†</td>
<td>0.025 ± 0.001†</td>
</tr>
<tr>
<td>Ischemia-reperfused, untreated (IR-0)</td>
<td>738 ± 57*†</td>
<td>1049 ± 105*†</td>
<td>0.117 ± 0.047*†</td>
<td>0.039 ± 0.01*</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.22-treated (IR-0.22)</td>
<td>585 ± 35*†</td>
<td>994 ± 109*†</td>
<td>0.34 ± 0.076*†</td>
<td>0.030 ± 0.002*†</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.55-treated (IR-0.55)</td>
<td>385 ± 35*†</td>
<td>709 ± 89*†</td>
<td>0.224 ± 0.06*†</td>
<td>0.030 ± 0.01*†</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.77-treated (IR-0.77)</td>
<td>401 ± 55†</td>
<td>299 ± 59†</td>
<td>0.204 ± 0.04†</td>
<td>0.026 ± 0.003*†</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-1.1-treated (IR-1.1)</td>
<td>485 ± 65*†</td>
<td>564 ± 71*†</td>
<td>0.31 ± 0.07*†</td>
<td>0.037 ± 0.003*†</td>
</tr>
</tbody>
</table>

* P < 0.01 vs. control, IR-0.55 groups or stabilization values; † P < 0.01 vs. IR-0 group; ‡ P < 0.01 vs. the 2-min corresponding reperfusion values

XO, xanthine oxidase plus xanthine dehydrogenase; GSH, reduced glutathione; IR, ischemia-reperfused organs

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**Fig. 2** Total xanthine oxidase (XO + XDH) and reduced glutathione (GSH) in the pancreas tissue. *P < 0.01 vs. controls; †P < 0.01 vs. all groups. Abbreviations: Control, continuously perfused pancreases; IR-0, ischemic-reperfused, untreated pancreases; IR-0.22, ischemic-reperfused, 0.22 mM mannitol-treated pancreases; IR-0.55, ischemic-reperfused, 0.55 mM mannitol-treated pancreases; IR-0.77, ischemic-reperfused, 0.77 mM mannitol-treated pancreases; IR-1.1, ischemic-reperfused, 1.1 mM mannitol-treated pancreases

**Fig. 3** Pulmonary perfusion pressure (lower plane) and ventilatory plateau pressure (upper plane). *P < 0.01 vs. control, IR-0.77 and IR-0.55 groups. Abbreviations: Control, continuously perfused pancreases; IR-0, ischemic-reperfused, untreated pancreases; IR-0.22, ischemic-reperfused, 0.22 mM mannitol-treated pancreases; IR-0.55, ischemic-reperfused, 0.55 mM mannitol-treated pancreases; IR-0.77, ischemic-reperfused, 0.77 mM mannitol-treated pancreases; IR-1.1, ischemic-reperfused, 1.1 mM mannitol-treated pancreases
weight throughout the experiments (Fig. 4). In contrast, the rest of the IR lungs progressively gained weight, starting at 5 min of reperfusion, ultimately doubling their original weight. This picture paralleled the WDRs of the lungs (Table 1): the IR-0 lungs had the highest WDRs and weight gain, whereas the IR-0.77 lungs had the lowest values among the IR groups. These derangements were associated with the final \( \text{Po}_{2}/\text{FiO}_2 \) values: the lowest values among the IR groups. These derangements were detected in the IR-0-attached lungs (Table 3).

Analysis of the IR-0 BAL volumes and the adjusted contents of such retrieved volumes proved to be abnormal as well. Amylase concentrations in the IR-0 and the IR-1.1 BALs were 2–4.5 times higher compared with the corresponding controls (Table 3). Total XO activity was high in all IR lungs, except for the controls and those attached to the IR-0.77 pancreases. These were in agreement with the IR-0 lungs' XO was 10–50% higher than in the controls and the IR-0.55 and IR-0.77 groups. The GSH content in these three groups varied within a range of 8–35%, and the values were similar between the controls and the IR-0.77 lungs.

### Discussion

This study was designed to investigate the effects of different doses of mannitol on pancreas IR-induced ALI in an

<figure>
<figcaption>Fig. 4 Intraexperimental lung weight gain. \(^*P < 0.03 \) vs. all other groups. Abbreviations: Control, continuously perfused pancreases; IR-0, ischemic-reperfused, untreated pancreases; IR-0.22, ischemic-reperfused, 0.22 mM mannitol-treated pancreases; IR-0.55, ischemic-reperfused, 0.55 mM mannitol-treated pancreases; IR-0.77, ischemic-reperfused, 0.77 mM mannitol-treated pancreases; IR-1.1, ischemic-reperfused, 1.1 mM mannitol-treated pancreases</figcaption>
</figure>

<figure>
<figcaption>Fig. 5 Lung tissue total xanthine oxidase (XO + XDH) and reduced glutathione (GSH) data. \(^*P < 0.01\) vs. the control, IR-0.55 and IR-0.77 groups; \(^†P < 0.01\) vs. all IR-treated groups; \(^‡P < 0.01\) vs. all IR-treated groups with the exception of the 0.22 and 1.1 mM groups. Abbreviations: Control, continuously perfused pancreases; IR-0, ischemic-reperfused, untreated pancreases; IR-0.22, ischemic-reperfused, 0.22 mM mannitol-treated pancreases; IR-0.55, ischemic-reperfused, 0.55 mM mannitol-treated pancreases; IR-0.77, ischemic-reperfused, 0.77 mM mannitol-treated pancreases; IR-1.1, ischemic-reperfused, 1.1 mM mannitol-treated pancreases
</figcaption>
</figure>

<table>
<thead>
<tr>
<th>Group</th>
<th>( \text{Po}_{2}/\text{FiO}_2 )</th>
<th>( \text{O}_{2}/\text{FiO}_2 )</th>
<th>Bronchoalveolar lavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>755 ± 45(^\dagger)</td>
<td>0.61 ± 0.05(^\dagger)</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>Ischemia-reperfused, untreated (IR-0)</td>
<td>545 ± 59(^*)</td>
<td>0.9 ± 0.07(^*)</td>
<td>90 ± 7(^*)</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.22-treated (IR-0.22)</td>
<td>611 ± 53(^*)</td>
<td>0.78 ± 0.06(^*)</td>
<td>75 ± 5(^*)</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.55-treated (IR-0.55)</td>
<td>667 ± 67(^\dagger)</td>
<td>0.65 ± 0.1(^\dagger)</td>
<td>43 ± 7(^\dagger)</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-0.77-treated (IR-0.77)</td>
<td>743 ± 49(^\dagger)</td>
<td>0.55 ± 0.08(^\dagger)</td>
<td>22 ± 4(^\dagger)</td>
</tr>
<tr>
<td>Ischemia-reperfused, mannitol-1.1-treated (IR-1.1)</td>
<td>689 ± 59(^*)</td>
<td>0.85 ± 0.09(^*)</td>
<td>39 ± 3(^*)</td>
</tr>
</tbody>
</table>

\(^*P < 0.01\) vs. controls or IR-0.55 group; \(^‡P < 0.01\) vs. IR-0 group

\( \text{XO} \) xanthine oxidase plus xanthine dehydrogenase; \( \text{GSH} \) reduced glutathione; \( \text{IR} \) ischemia-reperfused organs

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Lung

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Lung Tissue XO and GSH

Total XO activity in the lung tissues is displayed in Fig. 5. The IR-0 lungs' XO was 10–50% higher than in the controls and the IR-0.55 and IR-0.77 groups. The GSH content in these three groups varied within a range of 8–35%, and the values were similar between the controls and the IR-0.77 lungs.
isolated rat organ model. The findings repeated our previous results [9, 13]—that mannitol successfully attenuated the development of almost all detrimental phenomena in a bell-shaped dose-dependent manner in the lungs affected by the liver. The present report adds to the existing knowledge that mannitol is effective for pancreas-induced ALI as well. Ventilatory and perfusion parameters were effectively preserved when IR reperusates contained 0.77 (and less so 0.55) mM of mannitol. Both dosages also led to lung tissue GSH preservation and minimized XO-associated ROS activity far better than the other regimens. BAL indices were better in these two treatment groups than in the other IR groups. No protection was bestowed by the higher regimen, suggesting that other mechanism(s) might interfere with mannitol’s ROS-reducing capability in the lung [24].

There is abundant evidence of the buildup of ROS during reperfusion to explain the injury to the lung. The earlier documented large amounts of circulating XO associated with post-liver purine substrate and the observation of a high rate of oxygen consumption associated with low carbon dioxide washout during the initial phase of reperfusion that followed normothermic liver ischemia indicate a high rate of oxygen consumption, which was not available for metabolic purposes but rather for XO and the subsequent ROS generation (e.g., superoxide anion, hydrogen peroxide, or hydroxyl radical) [6, 9, 25, 26]. The high level of lactate found in the liver IR reperusate during a similar experiment [3] further supports this contention. The subsequent damage to the alveolocapillary membrane’s integrity that led to the reported increase in capillary filtration coefficient [3] closely correlates with the presently documented abnormally high BAL volume and content as well. Indeed, hydrogen peroxide (H$_2$O$_2$) induced pulmonary microvascular lesions when added to the isolated rabbit lung [25], and the XO-dependent O$_2$ radical was shown to cause epithelial cell injury [27]. Such data closely correlate with our findings of increased XO activity in the various reperusates. These BAL and the tissue solutes passed through the damaged alveolocapillary membrane in the IR-paired unprotected lungs [3, 9, 24]. The minimally affected ventilation, perfusion, wet gain, and BAL indices in the two middle-doses-treated lungs represent the salutary effect of mannitol on the lung, coinciding with low circulating XO and preserved GSH potency.

Besides its osmotic diuretic properties, mannitol possesses hydroxyl radical-scavenging properties [17, 28–31]. It also inhibits spontaneous aggregation of human platelets when subjected to anoxia and reoxygenation in vitro, and reduces the rate of production of H$_2$O$_2$ in patients undergoing coronary artery bypass grafting [32, 33]. It was demonstrated to provide neuroprotection by preventing both necrosis and apoptosis of cells after transient cerebral ischemia, and it protects ischemic muscles from reperfusion injury, as well as preventing liver-reperfusion-induced lung injury [16, 34–36]. Mannitol was shown to attenuate the harmful effects of hydroxyl radicals on cerebral arteries in cats, and to maintain the normal tonus of aortic rings that was otherwise altered by *OH* in rabbits and rats [17, 35, 37].

The attenuation of lung damage is not attributable to mannitol’s osmotic property because all solutions were below 308 mOsm. If one solution had exerted a distinctive osmotic effect, as was sometimes attributed to mannitol (e.g., in the kidney), it would have been the one containing 1.1 mM mannitol (it was to exclude this possible osmotic effect that the groups were treated with increasing different doses).

The abnormal changes in weight gain and BAL indices in the 1.1 mM group, as well as the moderately low XO activity and the relative preservation of GSH content in the lung tissue, together with the selective maintenance of the ventilatory parameters, could indicate an increased resistance to the therapeutic effect of this higher dose of mannitol. Koch et al. reported that the administration of a relatively high dose of N-acetyl-cysteine (NAC) to the rabbit was associated with a suppressed polymorphonuclear leukocyte oxidative burst (i.e., protective) activity, resulting in delayed *Escherichia coli* bacteria clearance from the blood and organs, thus enhancing organ colonization [38]. They suggested that this impaired potency of the granulocyte-dependent killing of the bacteria was due to the suppressed protective antioxidant properties of elements within the lung (resident leukocytes or macrophages [39]) because of the excessive dose of NAC that had been used. It has been shown recently that lung tissue could exhibit deficient antioxidant defense under the effect of a high NAC dose administration [40], depicting a picture quite similar to the present high doses of mannitol, as demonstrated in a previous study [30]. The data from these reports may explain the right side of the bell-shaped nature of mannitol’s therapeutic effects, where IR-1.1 lungs were much less protected from damage than the IR-0.55 or IR-0.77 lungs, even though the reperusate in the IR-1.1 group contained less XO than that in the IR-0.55 group. Nevertheless, these findings contradict previous reports that mannitol would not prevent remotely induced lung reperfusion damage [41, 42].

Clinical acute pancreatitis is diagnosed mainly by acute abdominal pain associated with a concomitant increase in serum amylase and lipase concentrations [19, 20]. Even though injury is usually mild, severe pancreatic damage develops in 20% of the patients, of whom 15–25% will die, many due to acute respiratory distress syndrome, especially among the critically ill [5, 43, 44]. Because the current
study documented tissue edema, abnormal BAL indices and hypoxia in association with postischemic pancreatitis, and because these phenomena were attenuated when XO activity was inhibited, these findings suggest the possibility of hindering ALI by the use of clinically applicable antioxidants, such as mannitol, at the first signs of lung function deterioration [3, 6, 8, 24, 45, 46].

The results of the present study confirm the long-standing hypothesis that the oxidant/antioxidant imbalance, i.e., XO increase and GSH decrease in any IR organ, is associated with pulmonary vascular and ventilatory injury. This occurred despite the two to three times lower total XO activity recorded in the pancreas compared to those found in the liver, for example [3, 6, 8], and the pancreatic effluent being diluted before entering the lungs. Amylase and lipase leaked out of all IR pancreases at similar magnitudes during reperfusion, independently of the mannitol regimen because of the IR-related cellular lyses. Nevertheless, the exposure of the normal lungs to the treated organs’ reperfusates did not end in ALI; in other words, amylase and lipase did not induce lung damage. Only the presence of sufficient XO activity and a relative shortage in GSH content in the IR circuits that were devoid of sufficient amounts of mannitol to counteract ROS activity were associated with lung damage.

GSH is an essential component in tissue oxidants-antioxidants balance [23, 24]. When exposed to remote organ IR, lungs pretreated with NAC were least damaged because of the augmented amount of the circulating GSH [24, 47]. GSH affords direct scavenging potentials, such as trapping $H_2O_2$, and consequently decreasing the production of the highly reactive hydroxyl radical [48]. It also is a natural scavenger of the superoxide anion, protecting the cellular protein thiol groups, which are essential for protein function and cellular integrity [22, 48, 49]. The data that emerged from the present study regarding the relationship between GSH and XO in the pancreas support the primary contention described previously [24, 50], i.e., that the imbalance of oxidants/antioxidants that follows remote organ IR may lead to lung edema, hypoxia, and increased vascular permeability in an otherwise normal lung. GSH leaked out of the damaged organs in the IR-0, 0.22, and 1.1 pancreases, but it was low in the same group’s BAL, probably because it had been consumed in the circuit as an antioxidant, as shown earlier [24]. Contrarily, the higher GSH contents in the BAL of the IR-0.55 and 0.77 lungs indicate a proportionately lower consumption because of the low oxidative activity in the circuit as a result of the antioxidant activity of mannitol. This explanation is supported by our previous publication of XO-related lung damage [9].

The attenuation of reperfusion injury in the normal lung induced by another organ IR (e.g., pancreas, liver) is one part of a complicated and important clinical issue. Although we do not attempt to extrapolate from the present results to clinical situations, these data do represent pertinent clinical features of ALI. The abnormal alveolo-capillary permeability and the subsequent fluid shift from the vasculature into the alveolar bed that lead to the clinical phenomenon of “wet lung” and edema are comparable to the herein depicted changes in lung weight gain and the abnormally retrieved volume and content of the BAL [15, 16]. Abnormally high perfusion and inspiratory pressures are paramount clinical indices of ALI that accompany aortic aneurysm repair or total vascular isolation [42, 51, 52]. From this clinical standpoint, the use of mannitol in cardioplegia to scavenge ROS during the procedure in humans was recently reported [53].

Conclusions

ALI is a frequent complication of damage to the pancreas. An early increase in alveolo-capillary membrane permeability can ensue, leading to increased respiratory plateau pressure associated with the passage of fluids and high molecular weight substances to the alveolar space, and thus to hypoxemia. Although the precise pathophysiology of clinical ALI occurring after pancreatitis is not completely understood, this experimental work in an isolated, double-organ animal model underlines the role of XO and ROS as being culprits in the promotion of ALI. Despite the narrow margin of efficacy, mannitol may still hold the promise of providing protection to the lung from reperfusion injury, and further studies are warranted to elucidate its yet unknown potentials during the complex chain of events that accompany acute lung reperfusion injury.

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References

Lung


