A Knack for NAC? Use of N-Acetylcysteine in Liver Transplant Ischemia-Reperfusion Injury

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Learning Objectives

1. Describe the pathophysiology of ischemia-reperfusion injury in liver transplant
2. Explain the mechanism of n-acetylcysteine’s hepatoprotective effects
3. Discuss current clinical issues regarding n-acetylcysteine usage for ischemia-reperfusion injury
4. Summarize currently available clinical evidence regarding n-acetylcysteine use for ischemia-reperfusion injury in liver transplant
I. Liver Transplantation

A. Cost of liver transplant
   i. Hospital transplant admission: $286,100
   ii. Total first year cost: $523,400

B. Indications for liver transplant: hepatitis C and alcoholic cirrhosis account for ≥ 50%

C. Number of liver transplants in 2009 and last five years
   i. United States: 6,291 and 38,484
   ii. Texas: 465 and 2,555
   iii. San Antonio: 86 and 627
   iv. University Hospital: 52 and 442

D. Graft availability: a valuable resource

E. Waiting list outcomes
   i. Nearly 4,000 candidates dropped out on the waiting list in 2008


Figure 1: US Liver Transplants Performed vs. Waiting List Candidates at End of Year 1999-2009

Figure 2: Registered Liver Transplant Candidates vs. Annual Drop-Out Rate

ii. As of December 2011, over 16,000 registered United Network for Organ Sharing (UNOS) candidates are waiting for an available liver

KEY POINTS
- Donor grafts a valuable commodity
- Cost of a liver transplant substantial
- Optimizing graft survival is essential
F. Significant changes in last twenty years

i. Improvement of organ procurement process
   a) Hypothermic preservation: increased available hypothermic time
   b) Preservation solutions: increased available hypothermic time

ii. Surgical techniques: improved to reduce ischemic times

iii. Immunosuppression: large movement from cyclosporine to tacrolimus

iv. MELD score: addition of MELD score to better stratify need for organ

v. Increased organ demand and improved techniques has led to increased use of less optimal grafts
   a) Donation after cardiac death donors (DCD)
      (1) 1999: 23 transplants from DCD performed
      (2) 2007: 307 transplants from DCD performed
   b) Steatotic livers: 13 to 26% of donor livers have hepatic steatosis

G. 2008 patient and graft survival in deceased donor liver transplants

i. 1-year: 88% patient, 84% graft

ii. 5-year: 74% patient, 68% graft

H. Acute postoperative complications

i. Acute rejection

ii. Biliary: strictures and leaks

iii. Vascular: hepatic artery and portal vein thrombosis

iv. Infections

v. Ischemia-reperfusion injury

I. Effects of postoperative complications

i. Initial poor function
   a) Graft dysfunction that can improve over time
   b) Can lead to graft failure

ii. Primary nonfunction
   a) Graft failure that will not improve acutely
   b) 4% of all liver transplants
   c) Lethal without re-transplantation

II. Ischemia-Reperfusion Injury (IRI)

A. IRI causes approximately 10% of early organ failure and can lead to an increased incidence of acute and chronic rejection

B. Risk factors for IRI

i. Recipient risk factor: increased MELD score

ii. Donor risk factors
   a) Age > 60
   b) Hepatic graft fat content > 30%
   d) Hospitalization > 5 days
   e) > 5 days pressor use
   f) Donation after cardiac death
   g) Prolonged ischemic time
      (1) Warm: low blood flow state
      (2) Cold: no blood flow state with hypothermic preservation techniques
C. Origin of injury

i. Donor death can create warm ischemia in poorly perfused states
ii. Hepatectomy from donor with hypothermic preservation creates cold ischemia
iii. Rewarming and reperfusion of graft post-anastomosis creates warm ischemia

D. Pathophysiology

- **Pathophysiologic effects**
  - Shift from aerobic to anaerobic metabolism
  - ATP metabolism leads to hypoxanthine byproduct that is converted to xanthine oxidase, increasing reactive oxygen species (ROS). This is exacerbated when O2 reintroduced upon reperfusion
  - Accumulation of anaerobic byproducts
  - Necrotic cell death

- **Response**
  - Cytokines and chemokines released
  - Glutathione stores depleted

- **Pathophysiologic effects**
  - Cytokines and chemokines activate Kupffer and dendritic cells, leading to hepatocyte phagocytosis
  - Increased production of superoxide, ROS, enzymes, and cytokines
  - Complement activation
  - Monocyte, neutrophil, and T-cell recruitment
  - Increased cellular adhesion via upregulation of cellular adhesion molecules

- **Response**
  - Inflammation amplification
  - Continued glutathione depletion

- **Pathophysiologic effects**
  - Complement activation of pro-apoptotic pathway
  - Vacuolization
  - Mitochondria and membrane damage

- **Response**
  - Cellular apoptosis

- **Pathophysiologic effects**
  - Accumulation of apoptotic cells
  - Platelet aggregation
  - Leukocyte entrapment
  - Decrease in nitric oxide (NO)

- **Response**
  - Sinusoid congestion
  - Vasoconstriction
  - Continued ischemia even after reperfusion

*Figure 3: Overview of Ischemia-Reperfusion Injury*
E. Management of IRI\textsuperscript{18,19}

i. Therapeutic goal: ameliorate effects of IRI

ii. Efficacy of potential treatment options is unclear

   a) IRI is a complex, pathophysiologic process that is not completely understood

   b) Certain molecular components have positive and negative effects

iii. Current graft preservation techniques to ameliorate effects of IRI are limited

   a) Hypothermic preservation
      
      (1) Slows cellular metabolism

      (2) Two techniques

      - Simple storage: graft flushed with and placed in preservation solution
      - Continuous perfusion: graft constantly perfused with preservation solution

   b) Preservation solution

      (1) University of Wisconsin (UW) solution

      - Standard of care for preservation solutions since the late 1980s
      - Components include: electrolytes, raffinose, glutathione, allopurinol, adenosine, dexamethasone, and hydroxyethyl starch

iv. Pharmacological interventions, specifically n-acetylcysteine

III. N-Acetylcysteine (NAC)

A. Use in other hepatic or ischemic injuries\textsuperscript{20,21}

i. Acetaminophen poisoning

   a) Mechanism of injury

      (1) Acetaminophen produces a toxic metabolite, N-acetyl-p-benzoquinonimine (NAPQI) that is normally inactivated by glutathione conjugation

      (2) Large quantities of acetaminophen deplete glutathione stores, leaving unconjugated NAPQI

      (3) NAPQI interacts with hepatic enzymes, causing damage

   b) Mechanism of action of NAC

      (1) Replenishes glutathione stores for NAPQI conjugation

      (2) Acts as a free radical scavenger to prevent further hepatocyte damage

   c) Regimen

      (1) Intravenous (Acetadote\textsuperscript{®} FDA-approved in 2004): 150 mg/kg (max 15 g) in D5W 200 mL over 60 minutes, then 50 mg/kg (max 5 g) in D5W 500 mL over 4 hours, then 100 mg/kg (max 10 g) in D5W 1000 mL over 16 hours

         - When commercial IV form (Acetadote\textsuperscript{®}) is unavailable, solution for inhalation has been used and infused through in-line 0.2 micron filter over 60 minutes\textsuperscript{22}

         - This is not USP 797-compliant

      (2) Oral (Mucomyst\textsuperscript{®}) diluted to 5% (FDA-approved): 140 mg/kg load, then 70 mg/kg q 4 hours X 17 doses

KEY POINTS

- NAC is FDA-approved to treat acetaminophen toxicity and used off-label for other ischemic injuries
- NAC’s proposed mechanism to treat IRI is multi-factorial
- NAC has been used to prevent IRI in liver transplants at University Hospital since 2002
- National shortage of NAC has prompted reevaluation of evidence for justification of its use
ii. Investigation for other indications have produced mixed results\textsuperscript{20,21}
   a) Hepatic ischemia (shock liver)
   b) Non-acetaminophen induced acute liver failure
   c) Contrast-induced nephropathy

B. Prevention of IRI in liver transplant
   i. Proposed mechanism of NAC for IRI\textsuperscript{21, 23-25}
      a) Replenishes glutathione stores under conditions for which the demand of glutathione is high
         (1) Glutathione conjugates with reactive oxygen species to form non-toxic metabolites
      b) Reduces the effect of toxic oxidative free radicals such as superoxides due to antioxidant properties as it is a rich source of sulfhydryl groups
      c) Enhances vasodilatory effect of nitric oxide (NO), improving hemodynamics and oxygen transport
         (1) Modulates the activity of inducible nitric oxide synthase (iNOS), an enzyme that metabolizes NO
      d) Decrease adhesion molecules such as intracellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM), subsequently reducing cellular adhesions

\textbf{Figure 4: Proposed Mechanism of Action of N-Acetylcysteine for Ischemia-Reperfusion Injury}

ATP= adenosine tri phosphate; NO= nitric oxide; ROS= reactive oxygen species
ii. Use at University Transplant Center
   a) Used routinely since 2002, at discretion of attending surgeon, for “high-risk” donors
      (estimated 60% of liver transplant recipients)
      (1) Regimen until August 2011
         • Inhalation form compounded for IV use: donor, 140 mg/kg IV prior to liver procurement; recipient, 140 mg/kg IV load, then 70 mg/kg IV q 4 hrs X 17 doses
         • Officially approved by P&T Committee in 2005 to continue use of compounded product because of Acetadote® cost (at the time estimated cost $7,500/course)
      (2) Nationwide shortage and increase in cost of inhalation formulation in April 2011 prompted change in regimen in August 2011
         • Recipient: Acetadote® 150 mg/kg in D5W 250 mL IV load in O.R., then 150 mg/kg in D5W 1000 mL IV (100 mg/kg over 4 hrs, then 50 mg/kg over 16 hrs)
         • Increase in cost and drug shortage prompted reevaluation of evidence supporting use for IRI prevention in liver transplant

C. Cost analysis of NAC

<p>| Table 1: University Hospital Cost Comparison for Available Dosage Forms of NAC |</p>
<table>
<thead>
<tr>
<th>Dosage Form</th>
<th>Mucomyst® Inhalation Generic formulation</th>
<th>Acetadote® Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Wholesale Price (AWP) per 6 g Vial</td>
<td>$12</td>
<td>$217</td>
</tr>
<tr>
<td>Inpatient Acquisition Cost per 6 g Vial</td>
<td>$4</td>
<td>$170</td>
</tr>
<tr>
<td>Inpatient Acquisition Cost for 150 mg/kg IV Load + 100 mg/kg IV + 50 mg/kg IV (80 kg Patient)</td>
<td>$16</td>
<td>$680</td>
</tr>
<tr>
<td>Inpatient Acquisition Cost for 140 mg/kg IV Load + 70 mg/kg IV X 17 Doses (80 kg Patient)</td>
<td>$72</td>
<td>$3,060</td>
</tr>
</tbody>
</table>
IV. Evidence

A. Initial studies in animal models were compelling

**Table 2: Summary of Animal Studies**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Animal</th>
<th>n</th>
<th>Intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fukuzawa N et al.</td>
<td>Porcine</td>
<td>16</td>
<td>NAC 150 mg/kg IV 1 hour before ischemia vs. NAC 150 mg/kg IV 20 minutes before reperfusion vs. placebo</td>
<td>NAC significantly improved glutathione stores and was associated with improved homeostasis, bile output, ATP regeneration, and survival</td>
</tr>
<tr>
<td>Koeppl TA et al.</td>
<td>Murine</td>
<td>16</td>
<td>NAC donor, 400 mg/kg; recipient, 400 mg/kg vs. placebo</td>
<td>Significantly reduced leukocyte adherence to sinusoid</td>
</tr>
<tr>
<td>Demir S et al.</td>
<td>Murine</td>
<td>43</td>
<td>NAC 500 mg/kg IV + pentoxifylline 50 mg/kg IV 15 minutes pre-ischemia vs. NAC 500 mg/kg IV 15 minutes pre-ischemia vs. pentoxifylline 50 mg/kg IV vs. placebo vs. control</td>
<td>NAC alone maintained glutathione levels</td>
</tr>
<tr>
<td>Nakano H et al.</td>
<td>Murine</td>
<td>--</td>
<td>NAC 150 mg/kg IV during cold ischemia time vs. placebo in steatotic and non-steatotic livers</td>
<td>Bile production and glutathione levels significantly increased and ALT significantly reduced in NAC group</td>
</tr>
</tbody>
</table>

B. Pilot studies in humans

i. Bromley et al.  
   a) Prospective, randomized, double-blind, placebo-controlled 
   b) 50 patients 
   c) Outcomes: INR, AST/ALT, cardiac index, oxygen delivery, length of hospital stay, graft function, morbidity, mortality, and number of rejection episodes at 1 month post-transplant 
   d) Intervention: NAC to recipient, 100 mg/kg IV, then 12.5 mg/kg/hr IV for 4 hours, then 6.25 mg/kg/hour x remainder of surgery 
   e) Results  
      (1) Significant differences in intraoperative cardiac index and oxygen delivery 
      (2) No statistically significant differences in other analyzed outcomes 

ii. Regueira et al.  
   a) Retrospective, case series 
   b) 62 patients 
   c) Outcomes: PT, bile production, AST/ALT, and number of rejection episodes 1 year post-transplant 
   d) Intervention: NAC to donor, 6 g IV one hour prior to liver removal 
   e) Results: Significant differences in PT, bile production, AST/ALT, and rejection episodes
<table>
<thead>
<tr>
<th>Design</th>
<th>Prospective, randomized, open-label, pilot study in Europe (Germany)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective</td>
<td>Evaluate the efficacy of NAC as a hepatoprotective agent in liver transplant recipients</td>
</tr>
<tr>
<td>Patient Population</td>
<td>● 60 transplants, 57 recipients from Jan 1996 to August 1997</td>
</tr>
<tr>
<td>Outcomes</td>
<td>● Macrocirculation: Doppler measurements of hepatic artery and portal vein flow</td>
</tr>
<tr>
<td>Interventions</td>
<td>● All transplants were standardized to local protocol</td>
</tr>
<tr>
<td>Statistics</td>
<td>● Student’s t-test for normally distributed data</td>
</tr>
<tr>
<td>Baseline Characteristics</td>
<td>● Baseline characteristics were similar except:</td>
</tr>
<tr>
<td>Results</td>
<td>● No statistically significant differences in all analyzed outcomes except:</td>
</tr>
<tr>
<td>Authors’ Conclusions</td>
<td>● Results of clinical pilot study confirm findings of animal models, justifying larger trials</td>
</tr>
<tr>
<td>Strengths</td>
<td>● Prospective, randomized trial</td>
</tr>
<tr>
<td>Limitations</td>
<td>● Single center, small patient population</td>
</tr>
<tr>
<td>Opinion</td>
<td>● The conditions assessed, with the exception of donor age, are not risk factors for IRI</td>
</tr>
<tr>
<td></td>
<td>● Greater incidence of primary nonfunction in placebo group that was non-statistically significant</td>
</tr>
</tbody>
</table>
Steib et al. 1998

<table>
<thead>
<tr>
<th>Design</th>
<th>Prospective, randomized, placebo-controlled study in Europe (France)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objectives</td>
<td>To evaluate the effects of NAC on intraoperative hemodynamics and postoperative graft function during liver transplantation</td>
</tr>
</tbody>
</table>
| Patient Population | • 60 patients  
• Underlying liver disease: 94% cirrhosis, 3% sclerosing cholangitis, and 3% familial amyloidosis  
• Child Pugh scores: 4 (6%) A, 31 (52%) B, 25 (42%) C |
| Outcomes | • Hemodynamic measurements before and after anesthesia administration, before infusion of NAC, 15 minute after revascularization, and 1 hour after hepatic artery anastomosis  
  o Heart rate (HR)  
  o Right arterial pressure (RAP)  
  o Mean arterial pressure (MAP)  
  o Pulmonary arterial pressure (MPAP)  
  o Pulmonary artery occlusion (PAO)  
  o Cardiac index (CI)  
• Macrocirculation via saturations 1 hour post-revascularization  
  o Oxygen delivery (VO\textsubscript{2})  
  o Oxygen consumption (DO\textsubscript{2})  
  o Oxygen extraction ratio (OER)  
• Postoperative graft function at 12, 24, 48, and 72 hours post-revascularization  
  o AST/ALT  
  o PT  
  o MEGX  
  o Factor V  
• Clinical outcomes  
  o 1-month mortality  
  o Retransplant  
  o ICU duration  
  o 1 year survival  
  o No statistical analysis performed, observational only |
| Interventions | • Treatment group (n=30): recipient, NAC 150 mg/kg IV over 30 minutes during reperfusion, then 50 mg/kg IV over 4 hours, then 100 mg/kg IV over 16 hours  
  • Diluent volume (D5W) not stated  
  • Placebo group (n=30): equivalent volume of D5W |
| Statistics | • Chi-squared test for qualitative variables  
• ANOVA for hemodynamic and tissue oxygenation variables  
• Nonparametric tests for graft function variables  
• p < 0.05 considered statistically significant |
| Baseline Characteristics | • Both groups were comparable with respect to demographic data and surgical considerations, including ischemic time |
| Results | • Differences between all hemodynamic parameters were nonstatistically significant, including hemodynamics (HR, MAP, RAP, PCWP, CI, SVRI, and PVRI), macrocirculation (VO\textsubscript{2}, DO\textsubscript{2}, OER, and lactates), and postoperative graft function (AST/ALT, PT, factor V, and MEGX) |

<table>
<thead>
<tr>
<th>Clinical Outcomes</th>
<th>NAC (n=30)</th>
<th>Placebo (n=30)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-month mortality</td>
<td>3 (10%)</td>
<td>0 (0%)</td>
<td>All had more progressive hepatic dysfunction. Two were re-transplanted and died, the other had CNS injuries</td>
</tr>
<tr>
<td>Retransplant</td>
<td>2</td>
<td>2</td>
<td>Both survived in placebo group, both died in NAC group</td>
</tr>
<tr>
<td>ICU duration (days)</td>
<td>6.4 ± 12</td>
<td>5.7 ± 9</td>
<td></td>
</tr>
<tr>
<td>1 year survival</td>
<td>26 (87%)</td>
<td>28 (93%)</td>
<td></td>
</tr>
</tbody>
</table>

Safety: No adverse events from NAC were noted
<table>
<thead>
<tr>
<th>Authors’ Conclusions</th>
<th>• NAC failed to show benefit over placebo on intraoperative hemodynamics, macrocirculation, and postoperative graft function</th>
</tr>
</thead>
</table>
| Strengths            | • Prospective, randomized trial  
• Standardization of operative conditions  
• Child-Pugh score was reported  
• Looked at a variety of hemodynamic factors to reduce potential confounders  
• Recorded operating room times shorter compared to Theis et al. trial |
| Limitations          | • Single center, small patient population  
• MELD score not defined/calculated as it did not exist at this point in time  
• Only donor characteristic assessed that is known to be a risk factor for IRI was age  
• Known inflammatory markers associated with IRI not assessed  
• <50% of recipients had a Child Pugh Class of C, not reflective of current patient population |
| Opinion              | • NAC does not seem to affect hemodynamics in liver transplant in recipients with chronic ESLD once the patient receives the graft |

C. Non-clinical outcomes in humans\textsuperscript{36-39}

i. Taut et al.\textsuperscript{36}

a) End outcome: expression of adhesion molecules 1 hours post-infusion, specifically ICAM, VCAM, sP-selectin, and sE-selectin  
b) Patients were treated with acetaminophen toxicity dosing of NAC or equivalent placebo  
   (1) Recipient, NAC in D5W 150 mg/kg IV loading dose, then 50 mg/kg IV for 4 hours, then 100 mg/kg IV for 16 hours (n= 9)  
   (2) D5W in the same volume (n=10)  
c) Results  
   (1) No significant difference between expression of adhesion molecules  
   (2) Increased elimination of selectins from the liver in NAC group indicating decreased activity of the adhesion molecules

ii. Weigand et al.\textsuperscript{37}

a) End outcome: expression of adhesion molecules, specifically ICAM, VCAM, sP-selectin, and sL-selectin, and glutathione marker α-glutathione S-transferase 24 hours post-infusion  
b) Patients were treated with acetaminophen toxicity dosing of NAC or equivalent placebo  
   (1) Donor graft, NAC 1,000 mg in Ringer’s solution was rinsed over the liver; recipient, NAC in D5W 150 mg/kg IV loading dose, then 50 mg/kg IV for 4 hours, then 100 mg/kg IV for 16 hours (n= 10)  
   (2) D5W in the same volume (n=10)  
c) Results  
   (1) NAC significantly decreased the adhesion molecules ICAM and VCAM and glutathione S-transferase levels
iii. Santiago et al.\textsuperscript{38} 
   a) End outcomes: extent of inflammation via anti-inflammatory cytokines, specifically IL-4 and IL-10 one hour postoperatively 
   b) Patients were treated with unique protocol dosing of NAC or equivalent placebo 
      (1) Recipient, NAC in D5W 100 mg/kg IV loading dose, then 50 mg/kg IV for 24 hours (n=25) 
      (2) D5W in the same volume (n=25) 
   c) Results: NAC significantly increased anti-inflammatory IL-4 at 5 minutes before and after reperfusion and IL-10 at 5 minutes before reperfusion 

D. Clinical outcomes in humans 
   i. Khan et al.\textsuperscript{39} 
     a) Evaluated if NAC administration to donor graft affected clinical outcomes in recipients 
     b) End outcomes: pathologic severity of IRI and incidence of rejection 1-year postoperatively 
     c) 4 patients excluded for fulminant hepatic failure (n=1) or postoperative hemorrhage (n=3) 
     d) Donor livers were treated prior to and during hypothermic preservation 
        (1) Donor, NAC in normal saline 150 mg/kg IV 15 minutes before cardiac arrest, then 
            NAC in citrate solution 75 mg/kg IV given via portal vein, then NAC in UW solution 1,000 mg was placed over the graft (n=9) 
        (2) No placebo solution was given; however, patients still received citrate solution and UW preservation solution (n=9) 
     e) Results: no significant differences between extent of IRI or episodes of rejection
### Design
Prospective, randomized, double-blind, placebo-controlled, ITT, in US (Pittsburgh)

### Objectives
- **Primary**: evaluate the efficacy of NAC for improving liver graft performance and lowering the incidence of post liver transplant acute kidney injury (AKI)
- **Secondary**: evaluate the effects of NAC on glutathione

### Patient Population
- 100 patients enrolled, 93 completed
- **Inclusion Criteria**: > 18 year old, first cadaveric orthotopic liver transplant, baseline serum creatinine < 2.5 mg/dl
- **Exclusion Criteria**: simultaneous other organ transplant, fulminant hepatic failure, pregnancy, or asthma

### Outcomes
- **Primary outcome**: 1-year graft and patient survival and 14-day incidence of AKI
- **Secondary outcome**: glutathione at baseline, anhepatic, 1 hour after NAC infusion, and 48 hours after NAC infusion

### Interventions
- **Treatment group** (n=50): recipient, NAC 140 mg/kg IV loading dose, then 70 mg/kg q 4 hours for a total of 12 doses (in NS)
- **Placebo group** (n=50): received equivalent volume of NS solution

### Statistics
- A sample of 100 patients was needed to detect a difference in post liver transplant AKI of 60%
- Logrank tests for baseline characteristic comparisons
- Survival analysis for time-to-event variables
- Multi-variable modeling for glutathione and risk factors
- P < 0.05 considered statistically significant

### Baseline Characteristics
- Both groups were comparable, including the following risk factors:
  - Number of extended criteria donors: 10 (NAC) vs. 9 (placebo)
  - MELD score: 14.4 ± 4.66 NAC vs. 14.1 ± 4.33 placebo
  - Number of patients who had veno-venous bypass: 45 (NAC) vs. 47 (placebo)
- The statistically significant exception was:
  - Duration of surgery 7.59 ± 1.71 hours NAC vs. 6.82 ± 1.36 hours placebo

### Results
- **Primary outcomes**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>NAC (n=50)</th>
<th>Placebo (n=50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-year graft survival (%)</td>
<td>74</td>
<td>80</td>
<td>0.54</td>
</tr>
<tr>
<td>1-year patient survival (%)</td>
<td>78.4</td>
<td>80.9</td>
<td>0.87</td>
</tr>
<tr>
<td>14-day incidence of AKI (%)</td>
<td>36</td>
<td>32</td>
<td>0.83</td>
</tr>
</tbody>
</table>

- **Post-hoc clinical outcomes**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>NAC (n=50)</th>
<th>Placebo (n=50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time on ventilator (days)</td>
<td>5.58 ± 9.79</td>
<td>4.30 ± 9.37</td>
<td>0.51</td>
</tr>
<tr>
<td>Duration of ICU stay</td>
<td>10.33 ± 11.60</td>
<td>8.84 ± 13.29</td>
<td>0.55</td>
</tr>
<tr>
<td>Duration of hospitalization</td>
<td>18.23 ± 15.49</td>
<td>19.00 ± 21.07</td>
<td>0.84</td>
</tr>
</tbody>
</table>
### Results

- **Secondary outcomes**

  ![Glutathione levels](image)

<table>
<thead>
<tr>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.49</td>
</tr>
<tr>
<td>Anhepatic</td>
<td>0.0001</td>
</tr>
<tr>
<td>1hr post-NAC</td>
<td>0.024</td>
</tr>
<tr>
<td>48hr post-NAC</td>
<td>0.56</td>
</tr>
</tbody>
</table>

  *NOTE: postoperative serum AST, ALT, INR, and total bilirubin were similar for 3 months*

- **Safety:** no adverse events were reported

### Authors’ Conclusions

- NAC was not effective in improving graft survival or reducing risk of mortality or AKI
- Only about half the patients achieved a significant rise in glutathione acutely, even though they received high-dose NAC

### Strengths

- Prospective, randomized, double-blind trial at University of Pittsburgh (pioneer US liver transplant center)
- Largest patient population to date
- Recipients’ MELD scores were analyzed
- Operative conditions were similar between groups
- Looked at clinical outcomes
- Defined extended donor criteria

### Limitations

- Single center, small patient population
- Sample size determined from incidence of post-transplant AKI and not powered for IRI
- Did not perform a subgroup analysis of high risk donors, although did note no statistically significant differences between groups
- MELD scores at time of transplant were low, not representative of current patient population
- Glutathione was only measured 1 and 48 hours post NAC-infusion

### Opinion

- Although NAC did increase glutathione levels, the effect was not sustained, which may explain why agent affects known components of pathophysiologic process but is unable to produce clinically significant differences
- Small sample size of high-risk donors makes it difficult to prove statistically significant outcomes like patient and graft survival
VI. Summary

A. IRI is a known complication of liver transplant that can lead to initial poor function and potentially further negative outcomes
B. Risk factors have been identified for IRI and majority of the risk is associated with the donor graft
C. Significant changes have occurred in liver transplantation since initial NAC trials
D. Current clinical evidence for NAC in IRI is inconsistent and not optimal in design
   i. Human and animal trials show positive results of NAC for improving markers of IRI
   ii. Human and animal trials show mixed results of NAC improving liver function
   iii. Human trials unable to show significant positive effects on clinical outcomes

VII. Conclusion

A. IRI is not totally preventable, but its negative effects may be reduced
B. Little evidence exists to show any strong clinical benefit of NAC for prevention of IRI
C. Given current data, NAC should not be used to prevent IRI in all liver transplant recipients
   i. More recent trials have demonstrated significant acute improvements in non-clinical outcomes such as anti-inflammatory markers and glutathione
   ii. However, evidence has failed to show significant improvements in clinical outcomes
      a) Markers of synthetic liver function
      b) Duration of ICU and hospital stay
      c) Patient and graft survival
D. NAC should only be used in recipients of high-risk donor grafts until stronger evidence is available
   i. No trials have been powered yet to analyze the use of NAC in recipients of high-risk donors
   ii. Human and animal trials suggest NAC may improve markers of IRI
   iii. Monetary cost of medication is insignificant when compared to the monetary cost of a liver transplant or commodity of the graft
   iv. No adverse effects of NAC were noted in any of the clinical trials
VIII. References


<table>
<thead>
<tr>
<th>Trial</th>
<th>Design</th>
<th>(n)</th>
<th>Treated</th>
<th>Dosing</th>
<th>Results (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromley, et al. 32</td>
<td>Randomized, Double-Blind, Placebo-controlled</td>
<td>50</td>
<td>Intraoperative</td>
<td>Treated only 100 mg/kg IV x 15 minutes → 12.5 mg/kg/hour IV x 4 hours → 6.25 mg/kg/hour x remainder of surgery</td>
<td>Significant differences in cardiac index and oxygen delivery intra-operatively</td>
</tr>
<tr>
<td>Regueira, et al. 33</td>
<td>Retrospective, Case Series</td>
<td>62</td>
<td>Preoperative</td>
<td>Donor only One hour prior to liver removal donor received 6g of NAC</td>
<td>Significant improvement in PT, bile production, and transaminases. Significantly fewer episodes of rejection in NAC group.</td>
</tr>
<tr>
<td>Santiago, et al. 38</td>
<td>Prospective, Randomized, Double-blind, Placebo-controlled</td>
<td>50</td>
<td>Intraoperative</td>
<td>Treated only NAC 100 mg/kg → 50 mg/kg via CIV x 24 hours</td>
<td>IL4 and IL10 Statistically increased</td>
</tr>
<tr>
<td>Khan, et al. 39</td>
<td>Prospective, Randomized, single-Center</td>
<td>22</td>
<td>Pre- and Perioperative</td>
<td>Treated only 150 mg/kg IV in normal saline 15 minutes before cardiac arrest → NAC in citrate solution 75 mg/kg IV → graft placed in mixture of NAC 1,000 mg and UW solution</td>
<td>No difference in severity of injury or incidence of rejection</td>
</tr>
<tr>
<td>Weigand, et al. 37</td>
<td>Prospective, Randomized, Open-label</td>
<td>20</td>
<td>Intraoperative</td>
<td>Treated only Liver was flushed with NAC 1,000 mg Then NAC 150 mg/kg loading dose → 50 mg/kg x 4 hours → 100 mg/kg x 16 hours</td>
<td>Adhesion molecules and α- GST were significantly lower</td>
</tr>
<tr>
<td>Taut, et al. 36</td>
<td>Prospective, Randomized, Placebo-Controlled, Blinded</td>
<td>19</td>
<td>Postoperative</td>
<td>Treated only 150 mg/kg loading dose → 50 mg/kg x 4 hours → 100 mg/kg x 16 hours</td>
<td>No significant differences</td>
</tr>
<tr>
<td>Bucuvalas, et al. 41</td>
<td>Prospective, Open label</td>
<td>25</td>
<td>Postoperative</td>
<td>Treated only 70 mg/kg over an hour post-reperfusion and q 12 hours x 6 days AND prostaglandin 0.4 mg/kg/hour via CIV x 6 days after first NAC dose</td>
<td>No significant differences in safety, patient/graft survival, length of stay, or liver enzymes</td>
</tr>
</tbody>
</table>