Paracetamol-Protein Adducts following Acute Paracetamol Overdose

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Paracetamol Adducts

Paracetamol

- Glucuronide (47 - 62%)
- Sulfate (25 - 36%)

Glutathione

- Reactive Intermediate (NAPQI)

- Cysteine & Mercapturic Acid Conjugates (5 - 8%)

Adducts:
- Paracetamol adducts
- APAP-adduct
- Protein adducts
- APAP-CYS
Paracetamol Adducts (APAP-CYS)

- Specific biomarker of paracetamol ingestion
- Studies in patients with:
  - hepatotoxicity ALT > 1000 IU/L
  - acute liver failure.
- APAP-CYS conc of 1.0 or 1.1 nmol/mL often used to identify these patients.

Paracetamol Adducts (APAP-CYS)

- Low levels APAP-CYS:
  - therapeutic doses of paracetamol
  - paracetamol overdose without hepatotoxicity

- Limited data:
  - Concentrations early in overdose (<24h).
  - whether initial APAP-CYS levels predict liver injury.

Australian Paracetamol Project (APP)

• **Aims:**
  • To characterise paracetamol adduct concentrations in patients with “large” paracetamol ingestions and/or paracetamol induced acute liver injury

• **Method:**
  • Multicentre prospective observational study.
  • Recruited through:
    • NSW Poison’s Information Centre
    • 3 clinical toxicology units in Australia
Australian Paracetamol Project (APP)

**Inclusion criteria:**
- Age ≥ 14 years
- Acute ingestions of IR paracetamol of:
  - ≥35g OR
  - Paracetamol concentrations ≥ 300mg/L (2000µmol/L) at any time OR
  - ALT/AST ≥ 500 U/L

**Definitions:**
1. ALT<50 U/L or unchanged from baseline
2. ALT 50 to 1000 U/L
3. ALT > 1000 U/L (hepatotoxicity)
Method

• 3 serum samples collected in the first 24h then as clinically indicated.

• Serum samples analysed by a previously reported HPLC method with electrochemical detection for APAP-cysteine derived by proteolytic cleavage of APAP adducts.

• Lower limit of quantification of 0.03nmol/mL
Results

• 53 patients recruited
• 335 serum samples
• Age: 24y (14 – 71y)
• Median dose of 48g (10 – 150g)
• Median time to IV NAC: 6.8h (1-77h) post-ingestion.
## Results

<table>
<thead>
<tr>
<th>ALT (IU/L)</th>
<th>&lt; 50</th>
<th>50 - 1000</th>
<th>&gt; 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>27</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td><strong>Dose (g; median; IQR)</strong></td>
<td>50 (40-60)</td>
<td>48 (35-50)</td>
<td>30 (20-50)</td>
</tr>
<tr>
<td><strong>Ethanol co-ingested</strong></td>
<td>6 (22%)</td>
<td>1 (14%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Time to NAC (h; median; IQR)</strong></td>
<td>5 (3 – 7)</td>
<td>6 (3 – 11)</td>
<td>22 (13 – 48)</td>
</tr>
</tbody>
</table>
First paracetamol concentration (> 4h)

Paracetamol concentration mg/l vs. time post ingestion (h)

Median time to first paracetamol concn: 6.3h (IQR: 4.7 – 15h)

Nomogram Line (150mg/L at 4h)

Double Nomogram Line (300mg/L at 4h)

ALT < 50 U/L
ALT 50 - 1000IU/L
ALT > 1000IU/L
ALT on presentation

 ALT U/L: 50 - 1000 IU/L
 ALT U/L > 1000 IU/L

ALT (IU/L)

Time post ingestion (h)
Paracetamol Adduct Plots

- APAP-CYS = 1.1 nmol/mL
- ALT < 50 U/L
- ALT 50-1000 U/L
- ALT > 1000 U/L

Paracetamol Adducts (nmol/mL) vs. Time post ingestion (h)
First APAP-CYS concentration >4h

Graph showing the relationship between time post ingestion (h) and paracetamol adducts (nmol/mL). The graph includes data points for different ALT levels:
- Green dots: ALT < 50 U/L
- Blue squares: ALT 50 - 1000 IU/L
- Red triangles: ALT > 1000 IU/L

A dashed purple line indicates APAP-CYS = 1.1 nmol/L.
First APAP-CYS concentration >4h (only patients presenting <24h)

Median APAP-CYS:
- ALT < 1000U/L: 0.33 (n=32)
- ALT > 1000U/L: 1.02 (n=11)

(IQR):
- ALT < 1000U/L: (0.22-0.48)
- ALT > 1000U/L: (0.69-1.86)
ROC: First APAP-CYS (< 24h post ingestion) value at predicting ALT > 1000IU/L

- Hepatotoxicity (ALT>1000U/L) n=11, control = 32
- ROC: AUC: 0.98 (P<0.0001)
- APAP-CYS = 0.58nmol/L:
  - Sensitivity = 100%
  - Specificity = 91%
ROC: Presentation within 24h of ingestion

- **Initial APAP-CYS**
  - AUC 0.98 (p < 0.0001)

- **Initial ALT**
  - AUC 0.86 (p < 0.0004)

- **AT (AST/ALT) x paracetamol concn**
  - AUC 0.67 (p = 0.076)

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**Example Calculations**

- APAP-CYS = 0.58nmol/L
- ALT = 50IU/L
- AT X APAP = 1500 mg/L x IU/L
- AT X APAP = 10000 mg/L x IU/L
ROC: Presentation within 8 hours

APAP-CYS
- AUC: 0.97
- p=0.002

ALT
- AUC: 0.83
- p=0.02

AT X APAP
- Multiplication product
- AUC: 0.58
- p=0.57
First APAP-CYS vs. peak ALT and INR (<24hr post ingestion) (n=44)

**Pearson r = 0.63**
(95%CI: 0.40 – 0.78)
P < 0.001

**Pearson r = 0.49**
(95%CI: 0.23 – 0.69)
P < 0.001
Limitations

• Small numbers developed hepatotoxicity with an initial normal ALT.

• Rely on patient history to determine time of ingestion.

• More patients required:
  • Presentation < 24h and developed hepatotoxicity or ALI
  • Higher peak INR’s OR meet transplant criteria
Conclusion

• Initial APAP-CYS > 0.58nmol/L <24h of ingestion is both sensitive and specific in predicting hepatotoxicity.
• APAP-CYS was more sensitive and specific than ALT or ATxAPAP <24h at predicting hepatotoxicity.
• Quicker assay required before APAP-CYS can be used in clinical practice.
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