Therapeutic drug monitoring (TDM) of thiopurine drugs

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ARUP laboratories
Topics

• Discuss the function of thiopurine drugs

• Thiopurine Drug Metabolism

• Pharmacogenetics of Thiopurine methyltransferase (TPMT)

• Describe analytical methods to support therapeutic drug management
Thiopurine drugs

Immunosuppressive antimetabolites

- Azathioprine (Imuran®)
- 6-Mercaptopurine (Purinethol®)
- Thioguanine (Tabloid®)

These compounds are analogs of hypoxanthine and guanine.
Thiopurine Drugs - Clinical Use

Acute lymphoblastic leukemia –

- Cancer in WBC
- Most common cancer in children
- 3,000 new cases / year (3.7-4.9 cases / 100,000) in children
- 98% remission after treatment

• Reference: www.stjude.org
Thiopurine Drugs - Clinical Use

Inflammatory Bowel Disease

- Autoimmune Disease
- 1.6 million Americans (80,000 children)
- 70,000 new cases / year
- Maintain remission 70-75%

• Reference: www.ccfa.org
Thiopurine Drugs - Clinical Use

Rheumatoid Arthritis

– Autoimmune Disease – tissues near joints
– 1.3 million adults (>18 yr)
– Incidence – 89 / 100,000 (65 – 74 yr);
  41 / 100,000 (30 – 65 yr)
– 50 – 60% remission after treatment

• Reference: www.cdc.gov
Metabolism of Thiopurine Drugs

Thiopurine Drugs - cytotoxicity

- 6-TGN resembles DNA nucleotides
- Incorporates into replicating DNA
  - Blocks proliferation (T-cells, B-cells)
- Inhibits purine de novo synthesis (6-MMPN)
- Promote immunosuppression
- Induce T-cell apoptosis
Is Thiopurine Therapy Effective in All Patients?

Table 2. Azathioprine tolerance: estimated proportions of patients

<table>
<thead>
<tr>
<th>Reported tolerance</th>
<th>Derma (n = 95)</th>
<th>Gastro (n = 92)</th>
<th>Rheum (n = 96)</th>
<th>All (n = 283)</th>
<th>P* (significant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well</td>
<td>69 (21)</td>
<td>75 (12)</td>
<td>59 (19)</td>
<td>67 (19)</td>
<td>&lt;0.001 (yes)</td>
</tr>
<tr>
<td>Reasonably well</td>
<td>17 (17)</td>
<td>13 (9)</td>
<td>22 (12)</td>
<td>17 (14)</td>
<td>&lt;0.001 (yes)</td>
</tr>
<tr>
<td>Poorly</td>
<td>14 (10)</td>
<td>13 (7)</td>
<td>19 (11)</td>
<td>15 (10)</td>
<td>&lt;0.001 (yes)</td>
</tr>
<tr>
<td>Missing responses</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

*ANOVA; P < 0.05.

Table 3. Estimated proportions of patients experiencing azathioprine side-effects

<table>
<thead>
<tr>
<th>Side-effect</th>
<th>Derma (n = 95)</th>
<th>Gastro (n = 92)</th>
<th>Rheum (n = 96)</th>
<th>All (n = 283)</th>
<th>P* (significant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>16 (16)</td>
<td>11 (10)</td>
<td>20 (18)</td>
<td>16 (15)</td>
<td>&lt;0.001 (yes)</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>0.1 (0.5)</td>
<td>2 (2)</td>
<td>0.3 (1.3)</td>
<td>0.6 (1.7)</td>
<td>&lt;0.001 (yes)</td>
</tr>
<tr>
<td>Neutropaenia</td>
<td>4 (6)</td>
<td>5 (4)</td>
<td>8 (11)</td>
<td>6 (8)</td>
<td>&lt;0.003 (yes)</td>
</tr>
<tr>
<td>Abnormal LFTs</td>
<td>9 (8)</td>
<td>5 (4)</td>
<td>10 (11)</td>
<td>8 (9)</td>
<td>&lt;0.001 (yes)</td>
</tr>
<tr>
<td>Missing responses</td>
<td>2</td>
<td>8</td>
<td>7</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

*ANOVA; P < 0.05.

Thiopurine S-methyltransferase (TPMT)

- Cytoplasmic s-methylation of thiopurines
  - 28 kDa, soluble protein; 245 amino acids
- Sites of Metabolism
  - Heart, Liver, Kidney, Intestines, Pancreas
  - RBC and WBC
- Exhibits genetic polymorphisms
  - May lead to adverse effects from thiopurine drug use

6-MMP 6-MP

6-MP → TPMT → 6-MMP
Distribution of TPMT Activity – Caucasian Population

Shaeffeler E et al, Pharmacogenetics 2004
Metabolism of Thiopurine Drugs

AZATHIOPRINE

6-MERCAPTOPURINE

TPMT

6-METHYL-MERCAPTOPURINE

Inactive metabolite

HGPRT

6-THIOURIC ACID

XO

6-THIOGUANINE

THIOGUANINE NUCLEOTIDES

Incorporates into DNA

Targeted Cytotoxicity
Metabolism of Thiopurine Drugs

- **AZATHIOPRINE**
  - **6-MERCAPTOPURINE**
    - **TPMT**
      - **6-METHYL-MERCAPTOPURINE**
    - **HGPRT**
      - **THIOGUANINE NUCLEOTIDES**
        - **6-THIOURIC ACID**
          - **Incorporates into DNA**
    - **Not in Bone Marrow**
      - **Inactive metabolite**
      - **Targeted Cytotoxicity**
Metabolism of Thiopurine Drugs

AZATHIOPRINE

6-MERCAPTOPURINE

HGPRT

THIOGUANINE NUCLEOTIDES

6-METHYL-MERCAPTOPURINE

Not in Bone Marrow

6-THIOURIC ACID

Incorporates into DNA

Inactive metabolite

Excessive Cytotoxicity
Bone Marrow Toxicity
TPMT Allele

Normal TPMT Allele

TPMT*1

- TPMT is ~30 kb long
- Consists of 10 exons
  - 8 of which encode for protein
- Patients with 2 normal TPMT alleles
  - normal high activity

Shaeffeler E et al. 2004, Pharmacogenetics
Genetic Mutations in TPMT Alleles

• Single Nucleotide Polymorphisms (SNPs)
  – Occur in the coding region of the gene
  – Occur at exon-intron splice sites
  – Can decrease the function of the enzyme

• Variable number tandem repeats (VNTRs)
  – Occur in the promotor region of the gene
  – Decrease function of the enzyme
  • Spire-Vayron et al, 1999; Roberts et al., 2008
Most Common Nonfunctional TPMT Alleles

- The four most common nonfunctional TPMT alleles account for >95% of low TPMT activity
- The most common allele variant found in the Caucasian population is the \textit{TPMT} *3A allele
- The most common allele variant found in the Asian and African population is the \textit{TPMT} *3C allele

Shaeffeler E et al. 2004, Pharmacogenetics
Mutations affect TPMT Activity

- Protein becomes unstable and undergo degradation
  - $TPMT^*2, *3A, *3B, *3C$ have enhanced proteolysis of variant proteins

- Decreased enzyme half-life
  - $TPMT^*1$ – **18 h** vs. $TPMT^*2$ and $TPMT^*3$ – **15 min**

- Variant alleles cause decreased enzyme activity
Dose Adjustments for TPMT Activity

**Phenotype**

<table>
<thead>
<tr>
<th>Low TPMT Activity</th>
<th>Intermediate TPMT Activity</th>
<th>High TPMT Activity</th>
<th>Very High TPMT Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3%</td>
<td>11%</td>
<td>89%</td>
<td>?</td>
</tr>
</tbody>
</table>

**Genotype**

<table>
<thead>
<tr>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
<th>Very High</th>
</tr>
</thead>
<tbody>
<tr>
<td>*2/*2, *2/*3A</td>
<td>*1/*2</td>
<td>*1/*1</td>
<td>?</td>
</tr>
<tr>
<td>*3A/*3A, *3C/*3C</td>
<td>*1/*3A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*3A/*3C, *3B/*3C</td>
<td>*1/*3C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Risk for Toxicity**

<table>
<thead>
<tr>
<th>Severe Bone Marrow Toxicity</th>
<th>Increased Risk</th>
<th>Low Risk</th>
<th>Low Risk High Risk?</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% of Standard Dose</td>
<td>30 - 70% of Standard Dose</td>
<td>Standard Dose</td>
<td>&gt; Standard Dose</td>
</tr>
</tbody>
</table>

Analytical Methods to support testing for thiopurine drugs
Testing Strategies to Access TPMT Activity

Phenotype

• Enzyme reaction – assess inactivation of thiopurine drug

• Quantification of thiopurine drug metabolites

Genotype

• Determines the allele pattern of a patient

• Primarily tests for 4 variant TPMT alleles –
  • TPMT*2, *3A, *3B, *3C
Phenotype Assay – TPMT Enzyme

- Add cosubstrates: 6-mercaptopurine & S-adenosylmethionine
- Incubate reaction @ 37 °C for 1 h
- Stop reaction with acid or heat:
- Prepare sample for HPLC or LC/MS/MS

- Remove plasma & WBC
- Lyse RBCs with water

6-MP + SAM → TPMT → 6-MMP

- Centrifuge
- Plasma → White blood cells & Platelets → Red blood cells

U/mL = nanomoles 6MMP

HPLC

Triple-Quad MS
Result Interpretation

**Low** (< 17 U/mL) – high risk of bone marrow toxicity with standard dosing regimens; dose reduction of 80-90% is recommended

**Intermediate** (17.0 - 23.9) – risk of bone marrow toxicity with standard dosing regimens; dose reduction by 20-50% may be necessary

**Normal** (24 - 44 U/mL) – low risk of bone marrow toxicity with standard dosing regimens

**High** (>44 U/mL) – risk of therapeutic failure due to excessive inactivation of these drugs. Patients may require a higher dose
Phenotype test - Thiopurine Drug Metabolites

- Test quantifies 6-TGN and 6-MMPN levels in whole blood

**Normal Reference Ranges**
- 6-TGN: 230-400 pmol/8x10^8 red blood cells
- 6-MMPN: <5700 pmol/8x10^8 red blood cells

![Chemical structures and pathways involved in the metabolism of thiopurines](image)

- **Azathioaprine (Imuran)**
- **HGPRT**
- **TPMT**
- **Leucopenia**
- **Hepatotoxicity**
Phenotype Test

Pros
• Less expensive than genotype test
• May be more informative than Genotype

Cons
• Enzyme function can be altered by:
  • NSAIDS can inhibit enzyme activity
  • Specimen mishandling can impact TPMT enzyme activity
  • Blood transfusion
    • Not accurately assessed in patients who receive RBC transfusion within 60 days of testing
Genotype TPMT assay

• PCR
  – Mini-sequencing assay
  – Fluorescence detection

• Detect the most common alleles
  – *TPMT*2 [238G>C]
  – *TPMT*3A [460G>A and 719A>G]
  – *TPMT*3B [460G>A]
  – *TPMT*3C [719A>G]
Genetic Test - PCR

**General OpenArray® Workflow**

The samples and master mix are mixed on an Intermediate Sample Plate (up to half of plate).

- **OA Master Mix**
- **DNA Samples**

5ul total volume

OpenArray® plates staked in case.

- Samples and 2x Master Mix loaded onto the OpenArray® plate.
- Lid affixed onto array case, filled with fluid, sealed, and loaded onto carrier.
- Plates are cycled and imaged on QS. (Optional: 9700 flat block for genotyping off-line cycling.)

**Analyze**

TaqMan Genotyper Software
Genotype Test

Pros

- Genotype test is not affected by –
  - Blood transfusions
  - Concomitant medications

Cons

- Assay cannot detect all of the nonfunctional TPMT alleles (>30)
- Wildtype or Normal alleles are inferred if none of the four non-functional alleles are detected
- Cannot distinguish between a heterozygote or a compound heterozygote
  - *1/*3A or *3A/*3C, *3A/*3B, *3B/*3C
Summary

• Phenotype assay may provide more clinical information
  • Detects rapid metabolizer phenotypes and impaired enzyme activity from rare genetic variants
  • Genotype test may not detect all rare mutations

• Genetic testing can be performed before and after thiopurine drug therapy
  • Consider testing if erythrocyte TPMT activity is abnormal
Take Home Message

• Test patients for TPMT status prior to therapy
  – Phenotype
  – Genotype

• Patients with low or absent TPMT activity
  – Avoid Thiopurine therapy or reduce the standard dose

• Genotype/phenotype tests to assess TPMT activity do not replace routine clinical monitoring