The Nobel Prize in Physiology or Medicine 1988

Development of azathioprine and 6MP in late 50s and early 60s

Gertrude B. Elion

Azathioprine and its metabolite 6-mercaptopurine are immunosuppressive agents with potent anti-inflammatory functions

Childhood leukemia

Organ transplantation

Autoimmune and chronic inflammatory diseases

Instanbul 5/2006
Azathioprine/ 6-MP in IBD

1962 First case report on 6-MP in IBD by R.H.D. Bean

38 year old patient with chronic active UC

300mg 6-MP: reduction of blood transfusions, X-ray: return of haustration, endoscopy: healing of colonic ulcers

50 mg 6-MP: return of clinical symptoms

300 mg followed by 100 mg 6-MP: Long-term remission over 80 weeks
Azathioprine/6MP in CD

Induction of remission  Maintenance of remission

Modified according to Lemann et al. 2006
Azathioprine for maintenance of steroid-induced remission in CD

N=63 patients with active CD

Prednisolone 1mg/kg with tapering

Clinical endpoint: Remission (CDAI<150)

Candy et al. Gut 1995
Children with newly diagnosed CD: Effects of 6MP and steroids

Early therapy ?: few controlled studies, Should be considered in selected patients

Markowitz et al. Gastroenterology 2000
6-MP for prevention of postoperative relapse

- 6-MP (50mg/d!)
- 5-ASA (3g/d)
- Placebo
- 2 year study
- 131 patients
- 56% dropout rate

Clinical rec: 6-MP vs placebo, p=0.045
5-ASA vs placebo, p=0.15

Hanauer et al. Gastroenterology 2004
Azathioprine in prevention of postoperative relapse

- Azathioprine (2mg/kg/d)
- 5-ASA 3g/d
- N=142 patients
- Azathioprine/5-ASA
- Effectiveness?
- Power of the study?

Ardizzone et al. Gastroenterology 2004
Combination therapy of Azathioprine/6MP and infliximab

<table>
<thead>
<tr>
<th>Remission (CDAI&lt;150) &amp; off steroids</th>
<th>Placebo (n=58)</th>
<th>Infliximab (n=57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>week 12</td>
<td>P&lt;0.0001</td>
<td>38%</td>
</tr>
<tr>
<td>week 24</td>
<td>P=0.003</td>
<td>29%</td>
</tr>
<tr>
<td>week 52</td>
<td>P=0.04</td>
<td>22%</td>
</tr>
</tbody>
</table>

W 0, 2, 6 + AZA/MP

5 mg/kg W 0, 2, 6 + AZA/MP
Duration of therapy?

- % of relapse at 2 yr
  - **stopped**
  - **maintained**

- Duration of remission on azathioprine (years)
  - 0-1: 150
  - 1-2: 99
  - 2-3: 57
  - 3-4: 35
  - 4-5: 24
  - >5: 12

Bouhnik et al. Lancet 1996
Double blind placebo controlled azathioprine withdrawal study

Non inferiority hypothesis not rejected: p=0.19

Lemann et al. 2006
Long term azathioprine therapy in IBD

Almost 1200 Patients
818 CD
358 UC
Holtmann et al.
DigDisSci 2006

Instanbul
Mechanism of action

Metabolism of azathioprine:

- azathioprine
- 6-mercaptopurine
- 6-thiouric acid
- 6-methyl-mercaptopurine
- 6TGN

- wide interindividual differences in TPMT activity (Lennard, 2000)
- low TPMT activity -> high sensitivity to azathioprine (Lennard, 2000)
- immunosuppressive effects of azathioprine (Colombel et al., 2000)
- random incorporation into DNA might be responsible for cytotoxic effects of azathioprine (Lennard, 2000)
The antiproliferative effect requires high dosages of 6-MP

Relative cell number/ CFSE labeling

PBL cultured for 4 days

Azathioprine mediated effects on T lymphocytes:

Co-stimulation through CD28

*In vitro* treatment with azathioprine

Human T lymphocytes were isolated from blood

Azathioprine interacts with the CD28 mediated intracellular pathway and thereby causes apoptosis

5 days

untreated

azathioprine

Azathioprine mediated induction of apoptosis in T lymphocytes
6-MP responsiveness correlates with the induction of apoptosis

Tiede et al. J. Clinical Invest. 2003
Further azathioprine mediated effects on T lymphocytes

Co-stimulation through CD28

*In vitro* treatment with azathioprine

T lymphocytes

Azathioprine reduced the number of intercellular contacts between T cells and APCs

Azathioprine interacts with the CD28 mediated intracellular pathway and thereby blocks T cell-APC conjugation

*Poppe et al. J. Immunol. 2006*
Specificity of 6-ThioGTP binding to Rac1

6-thioguanine-triphosphate (6-Thio-GTP) versus Ras and recombinant Rac1

Measure bound radiolabelled GTP

Relative GTP-binding (% of control)

Thio-GTP (µM)
A molecular mechanism of action of azathioprine

CD28

Rac1/2

vav

Rac-thioGTP

STAT3

IκB

NF-κB

Bcl-xL

azathioprine

ERM

Inhibition of T cell-APC conjugates

Induction of apoptosis
Azathioprine blocks vav activity on Rac1

- 6-Thio-GTP
- CD28
- Rac1-Thio-GDP
- Rac1-Thio-GTP

Apoptosis, Inhibition of T cell-APC conjugation
Diagnostic implications: Metabolism of azathioprine/6-MP

- Azathioprine/6-MP
- 6-MP
- 6-TIMP
- HPRT
- IMPDH
- GMPS
- 6-TGMP
- TPMT
- 6-TGDP
- 6-TGTP
- 2´-deoxy-6-TGDP
- 2´-deoxy-6-TGTP
- 6-MTGN
- NDPK
- Rac1
- ND PK
- Proliferation
- DNA
- Apoptosis

Metabolism of azathioprine/6-MP
### Study population (n= 110)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>mean</th>
<th>%</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45</td>
<td></td>
<td>18-72</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>14</td>
<td></td>
<td>0-49</td>
</tr>
<tr>
<td>Diseased area of the gut</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small and large bowel</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large bowel</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small bowel</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fistulas</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indication for azathioprine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refractory disease</td>
<td>69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous surgery</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of therapy (years)</td>
<td>3.1</td>
<td></td>
<td>0-14</td>
</tr>
<tr>
<td>Dosage of azathioprine (mg)</td>
<td>123</td>
<td></td>
<td>25-250</td>
</tr>
<tr>
<td>Dosage per kilogram weight</td>
<td>1.9</td>
<td></td>
<td>0.7-2.5</td>
</tr>
</tbody>
</table>
Design of the study

Blood was taken on clinical visits

The following parameters were determined:
TGN, TGMP, TGDP, TGTP, TPMT, TGTP/ TGMP+TGDP+TGTP

Clinical parameters were determined prospectively including
Steroid use, infliximab demand, CDAI, and number of flares

Patients were followed for a period of 6 months

Outcome: in 20 patients no metabolites could be measured, 5 patients were lost during follow-up, all remaining patients could be further analyzed
6-TGN levels correlate with TGTP and TGDP

\[ r = 0.15 \quad p = 0.31 \]
\[ r = 0.78 \quad p < 0.0001 \]
\[ r = 0.88 \quad p < 0.0001 \]
6-TGN levels correlate with TGTP plus TGDP levels

\[ r = 0.89 \]
\[ p < 0.0001 \]

\[ r = 0.79 \]
\[ p < 0.0001 \]
TGDP but not TGTP levels correlate with drug dosage

dose/ kg body weight

\[ r = 0.30 \]
\[ p = 0.08 \]

\[ r = 0.39 \]
\[ p = 0.007 \]
TGDP but not TGTP levels correlate with drug dosage

\[ r = 0.22 \]
\[ p = 0.17 \]

\[ r = 0.25 \]
\[ p = 0.13 \]
Correlation of 6-TGN levels with clinical parameters

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>TGN</th>
<th>TGDP</th>
<th>TGTP</th>
<th>R (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGN&gt; 100</td>
<td>23</td>
<td>184+/-16</td>
<td>32+/-7</td>
<td><strong>132+/-12</strong></td>
<td><strong>81+/-3</strong></td>
</tr>
<tr>
<td>TGN&lt; 100</td>
<td>24</td>
<td>59+/-4</td>
<td>9+/-2</td>
<td>55+/-7</td>
<td>84+/-2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Resp.</th>
<th>Flares</th>
<th>INF</th>
<th>TPMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGN&gt; 100</td>
<td>0.42+/-0.1#</td>
<td>1.3+/-0.2</td>
<td>0.3+/-0.1</td>
<td>49+/-2</td>
</tr>
<tr>
<td>TGN&lt; 100</td>
<td>0.16+/-0.1</td>
<td>1.6+/-0.5</td>
<td>0.4+/-0.1</td>
<td>48+/-1</td>
</tr>
</tbody>
</table>

# P=0.05
* P<0.05
** P<0.01

INF= infliximab use

### TGN> 100 pmol/8x10⁸ RBC

<table>
<thead>
<tr>
<th></th>
<th>TGN</th>
<th>TGDP</th>
<th>TGTP</th>
<th>R (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGN&gt;100 R&gt; 85</td>
<td>194+/-21</td>
<td>18+/-2*</td>
<td>151+/-15*</td>
<td>89+/-1</td>
</tr>
<tr>
<td>TGN&gt;100 R&lt; 85</td>
<td>175+/-24</td>
<td>46+/-12</td>
<td>113+/-18</td>
<td>72+/-4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Resp. Flares</th>
<th>INF</th>
<th>TPMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGN&gt;100 R&gt; 85</td>
<td>0.81+/-0.1*</td>
<td>0.27+/-0.1</td>
<td>48+/-3</td>
</tr>
<tr>
<td>TGN&gt;100 R&lt; 85</td>
<td>0.36+/-0.1</td>
<td>0.41+/-0.1</td>
<td>49+/-4</td>
</tr>
</tbody>
</table>

### TGN< 100 pmol/8x10⁸ RBC

<table>
<thead>
<tr>
<th></th>
<th>TGN</th>
<th>TGDP</th>
<th>TGTP</th>
<th>R (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGN&lt;100 R&gt; 85</td>
<td>59+/-5</td>
<td>7+/-1*</td>
<td>55+/-6</td>
<td>88+/-1</td>
</tr>
<tr>
<td>TGN&lt;100 R&lt; 85</td>
<td>58+/-9</td>
<td>14+/-3</td>
<td>55+/-12</td>
<td>76+/-4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Resp. Flares</th>
<th>INF</th>
<th>TPMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGN&lt;100 R&gt; 85</td>
<td>0.2+/-0.1</td>
<td>0.31+/-0.1</td>
<td>48+/-1</td>
</tr>
<tr>
<td>TGN&lt;100 R&lt; 85</td>
<td>0.4+/-0.2</td>
<td>0.50+/-0.2</td>
<td>51+/-3</td>
</tr>
</tbody>
</table>
Hypothetical model of azathioprine responsiveness

- High 6-TGN
- azathioprine (6-MP)
- High 6-TGDP → low 6-TGTP
- Rac1 → apoptosis → response
- Rac1 blockade
- low 6-TGDP → high 6-TGTP
- High 6-TGN
Summary

- Novel assays to measure TGMP, TGDP and TGTP were established
- TGDP and TGTP appear to be the main metabolites within 6TGN
- TGDP rather than TGTP levels correlate with azathioprine dosage
- Patients with low TGTP levels seem to respond poorly to therapy even in the presence of high 6TGN levels
- Patients with high TGDP levels have a higher steroid demand than those with low TGDP levels
Future goals

azathioprine
6-MP

6-TGN

6-TG

6-MP → 6-TIMP

HPRT

IMPDH

GMPS

NDPK

6-TGMP → 6-TGDP

HPRT

TPMT

Rac1

6-MTGN

2´-deoxy-6-TGDP

2´-deoxy-6-TGTP

DNA

proliferation

apoptosis
Therapeutic implications: Steric modelling of ThioGTP

Cyan = switch 1 region of Rac1 (residues 27-35)
Magenta = switch 2 region of Rac1 (residues 59-71)
Yellow = contact area of vav1 with Rac1
Identification of 6-Thio-GTP analogues

Synthesis of 5 main groups of derivatives:

**Group A**

**Group B**

**Group C**

05A-0
05A-1
05A-2
05A-3

05B-0
05B-1
05B-2
05B-3

C1-C17
Rapid induction of apoptosis by a 6-Thio-GTP analogue

Day 3 of cell culture

untreated

azathioprine

NEW

Rapid induction of apoptosis by a 6-Thio-GTP analogue

Day 3 of cell culture

untreated

azathioprine

NEW
Summary:

Azathioprine induces T cell apoptosis

Azathioprine works by blocking CD28 signaling via Rac1 (not by inhibition of proliferation)

Diagnostic implications: Monitoring of TGDP and TGTP

Future therapies: 6ThioGTP analogues
Thank you
## Side effects of azathioprine/6-MP in IBD

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>Present</th>
<th>Goldstein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 396)</td>
<td>(n = 347)</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>3.3 %</td>
<td>4.3 %</td>
</tr>
<tr>
<td>Bone marrow toxicity</td>
<td>2.0 %</td>
<td>1.2 %</td>
</tr>
<tr>
<td>Allergy</td>
<td>2.0 %</td>
<td></td>
</tr>
<tr>
<td>Hepatitis</td>
<td>0.3 %</td>
<td>0.3 %</td>
</tr>
<tr>
<td>Infections</td>
<td>7.4 %</td>
<td>1.4 %</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>3.1 %</td>
<td>1.2 %</td>
</tr>
</tbody>
</table>

In a retrospective study in 155 pregnant patients no increased risk for abortions and malformations was found.
Liver toxicity

- Less common but more serious liver toxicity: VOD, nodular regenerative hyperplasia
- True incidence not well understood