Mechanisms of pyrazinamide toxicity

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Pyrazinamide
(pyrazinoic acid amide
pyrazine carboxylamide)
Introduction

• Side effects profile

• Mechanisms

• Future research
PZA toxicity

- Adverse events:
  - Livertoxicity
  - Gastro-intestinal: anorexia, nausea and vomiting
  - Arthralgia
  - Hypersensitivity reactions: urticaria, pruritis, skin rashes, photosensitization, sideroblastic anemia
  - Effects on blood clotting mechanism or vascular integrity?
PZA toxicity

- Adverse events:
  - Liver toxicity (DILI, ATDH, ADIH)
  - Gastro-intestinal: anorexia, nausea and vomiting
  - Arthralgia
  - Hypersensitivity reactions: urticaria, pruritis, skin rashes, photosensitization, sideroblastic anemia
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PZA toxicity

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[Toxic reactions during treatment with pyrazinamide in pulmonary tuberculosis]

[Article in Polish]

Radecki A, Kanwiszer H, Krzanowska E, Donderowicz A.

PMID: 4877969 [PubMed - indexed for MEDLINE]
Tb Drug Induced Liver Injury

- 5-10% of all patients treated for active TB
Review

Antituberculosis drug-induced hepatotoxicity: Concise up-to-date review

Alma Tostmann, Martin J Boeree, Rob E Aarnoutse, Wiel C M de Lange, Andre J A M van der Ven and Richard Dekhuijzen

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American Thoracic Society Documents

An Official ATS Statement: Hepatotoxicity of Antituberculosis Therapy


This official statement was approved by the ATS Board of Directors, March 2006
PZA metabolism

- Metabolized and detoxified in the liver
- Excreted renally
Metabolism of PZA

Figure 1. Metabolism of pyrazinamide (Liacroix et al., 1989).
Table 1. Pharmacokinetic parameters of PZA and its metabolites after a single oral dose of PZA 27 (3.4) mg·kg\(^{-1}\) (mean (SD))

<table>
<thead>
<tr>
<th></th>
<th>(C_{\text{max}}) (mg·l(^{-1}))</th>
<th>(t_{\text{max}}) (h)</th>
<th>(t_{1/2\alpha}) (h)</th>
<th>(t_{1/2\beta}) (h)</th>
<th>MRT (h)</th>
<th>AUC (mg·l(^{-1}·)h)</th>
<th>CL ml·min(^{-1})</th>
<th>V l·kg(^{-1})</th>
<th>CL(_R) ml·min(^{-1})</th>
<th>Ae 0-72 h (% dose)</th>
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<tbody>
<tr>
<td>PZA</td>
<td>38.7 (5.9)</td>
<td>1</td>
<td>1.6</td>
<td>9.6</td>
<td>11.6</td>
<td>520</td>
<td>61</td>
<td>0.71</td>
<td>1.9</td>
<td>3.2</td>
</tr>
<tr>
<td>PA</td>
<td>4.5 (0.9)</td>
<td>4.9</td>
<td>-</td>
<td>11.8</td>
<td>15.9</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>106</td>
<td>33.2</td>
</tr>
<tr>
<td>5-OH-PZA</td>
<td>1.8 (0.3)</td>
<td>4.0</td>
<td>-</td>
<td>9.7</td>
<td>13.6</td>
<td>35</td>
<td>-</td>
<td>-</td>
<td>197</td>
<td>19.7</td>
</tr>
<tr>
<td>5-OH-PA</td>
<td>0.58 (0.17)</td>
<td>4.2</td>
<td>-</td>
<td>15.3</td>
<td>17.2</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>464</td>
<td>16.5</td>
</tr>
<tr>
<td>PZU</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Notes:
- PZA: Pyrazinamide
- PA: Pyrazinoic acid
- 5-OH-PZA: 5-OH-Pyrazinamide
- 5-OH-PA: 5-OH-Pyrazinoic acid
- PZU: Pyrazinuric acid
PZA toxicity mechanism
Section 11: Toxicological Information

Routes of Entry: Inhalation. Ingestion.

Toxicity to Animals:
LD50: Not available.
LC50: Not available.

Chronic Effects on Humans:
MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells.
DEVELOPMENTAL TOXICITY: Classified Reproductive system/toxin/female [POSSIBLE].

Other Toxic Effects on Humans: Slightly hazardous in case of skin contact (irritant), of ingestion, of inhalation.

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans:
May affect genetic material.
May cause adverse reproductive effects (maternal fertility) based on animal data.
May cause cancer (tumorigenic) based on animal data.
Found to be excreted in breast milk in small amounts.

Special Remarks on other Toxic Effects on Humans:
Acute Potential Health Effects:
Skin: May cause skin irritation. May cause photosensitivity
Eyes: Dust may cause eye irritation. May cause painful sensitization to light
Inhalation: Dust may cause respiratory tract irritation
Ingestion: May cause digestive tract irritation. May affect behavior, liver, cardiovascular system.

The toxicological properties of this substance have not been fully investigated.

Source: DrugBank
PZA toxicity mechanism

• PZA liver toxicity is dose dependent

*Tubercle 59* (1978) 13–32

THE HEPATIC TOXICITY OF ANTITUBERCULOSIS REGIMENS CONTAINING ISONIAZID, RIFAMPICIN AND PYRAZINAMIDE

D. J. Girling

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• Maybe it is not....
FIG. 2. Relationship between proportions of patients receiving pyrazinamide monotherapy who developed adverse events and cumulative pyrazinamide dose. Dose-dependent toxicity was driven by arthralgia. For the two patients who had hepatotoxicity, one received 2.7 g/day and the other received 3.8 g/day of pyrazinamide.
Hepatotoxicity of Pyrazinamide
Cohort and Case-Control Analyses

Kwok C. Chang¹, Chi C. Leung¹, Wing W. Yew², Tat Y. Lau¹, and Cheuk M. Tam¹


AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject
Relatively little is known about the hepatotoxicity of pyrazinamide. Previous controlled trials that suggested non-significant hepatotoxicity for pyrazinamide were neither designed nor sufficiently powered for evaluating hepatotoxicity.

What This Study Adds to the Field
Adding pyrazinamide to isoniazid and rifampin increases the risk of hepatotoxicity appreciably.
Idiosyncratic type B drug reaction: what is it?

Idiosyncratic drug reactions: the reactive metabolite syndromes

THE LANCET • Vol 356 • November 4, 2000

Sandra R Knowles, Jack Uetrecht, Neil H Shear

• Unpredictable
• Not dose dependent
• Hypersensitivity/immune system involvement?
• Probably due to reactive metabolites
• Mechanisms remain elusive
• Definitions not clear
Only idiosyncratic drug reaction when a danger signal occurs (cell death products)
Recent studies on PZA toxicity 1

• Question: what is the exact role of interactions with other TB drugs existing (INH, RIF)

Isoniazid and its toxic metabolite hydrazine induce in vitro pyrazinamide toxicity

Alma Tostmann a,b,*, Martin J. Boeree b, Wilbert H.M. Peters c, Hennie M.J. Roelofs c, Rob E. Aarnoutse d, André J.A.M. van der Ven e, P.N. Richard Dekhuijzen a

Interactions matter

• E.g. RIF induces CYP\textsubscript{450}\textsubscript{3A4}, therefore increases formation of hepatotoxic hydrazine

• In PZA interactions not described, and poorly investigated
Isoniazid metabolism

Indirect hydrazine formation

Acetylisoniazid $\xrightarrow{\text{Acetylation}}$ Isoniazid $\xrightarrow{\text{Hydrolysis}}$ Acetylhydrazine $\xrightarrow{\text{Hydrolysis}}$ Diacetylhydrazine $\xrightarrow{\text{Acetylation}}$

Direct hydrazine formation

Isoniazid $\xrightarrow{\text{Hydrolysis}}$ Isonicotinic acid $\xrightarrow{\text{Hydrolysis}}$ Hydrazine $\xrightarrow{\text{Acetylation}}$

Isonicotinyl glycine

Legend:
- - - - leads to hydrazine formation
- - - - leads to formation of non toxic metabolites
Enzymes involved in toxicity

Best known with isoniazid

- CYP1A1, 1A2, 2B1 and CYP2E1 are involved in isoniazid and/or hydrazin toxicity
- Cell studies with hepatoma cells (HepG2):
  - Induction of CYP1A1 and 1A2: no effect on INH toxicity
  - Enzymeinduction with rifampin: increase of INH toxicity

INH $\rightarrow$ both induction as inhibition of CYP2E1 (dependent of concentration)

Hydrazin induces own toxicity. Repeated exposure $\rightarrow$ more toxicity.
**Human liver microsomes:**
INH → inhibition of CYP2C19, CYP3A4, CYP1A2, CYP2A6, CYP2E1 en CYP2C9.
(microsomes: membranes within the cell on which the enzymes are attached).

**Study in humans:**
INH → inhibition of CYP2E1.

**Genetic polymorphism studies:**
- N-acetyltransferase 2: slow acetylators → higher probability of developing hepatoxicity.
- Glutathion S-transferase: null-mutation → higher probability of developing hepatoxicity.
- CYP2E1: c1/c1 genotype → higher probability of developing hepatoxicity.
Questions *in vitro* studies

**Mechanism of hepatotoxicity and interactions.**

- Which 1st line TB drugs *interact* with each other in terms of toxicity

- **2RZ** (prophylactic treatment) gives severe hepatotoxicity even as compared with the standard treatment of active TB (HRZE). Can we confirm *in vitro*?
**In vitro: the model**

**The model:**
- Cell model with human hepatoma cells (**HepG2**).
- In 96-wells plates:

**Day 1:** incubate 60.000 cells/well (24 hr).

**Day 2:** 24 hrs exposure to non-toxic concentrations of INH, HZ, RIF or PZA.

**Day 3:** 24 hrs exposure to increasing concentrations INH, HZ, RIF and PZA.

**Day 4:** with a cytotoxicity-assay (WST= Water soluble Tetrazolium salts) the cell survival is assessed → ‘toxicity curve’.

Every dose step test 4 times repeated in triplicate.
In vitro: WST cytotoxicity assay

WST-protocol:

Increasing concentrations of INH, Hz, RIF, PZA
After 24 hrs: cell survival determined with WST.

WST = orange coloured which changes to dark orange by viable cells
Cell survival quantified with ELISA-reader
IC50 = concentration of 50% cell death

Pretreatment with other TB drugs → enzyme induction? → effect on toxicity of TB-drugs?

**Decreased IC50** (shift to the left): A lower concentration causes an increase of cell death → Increased toxicity.

**Increased IC50** (shift to the right): An increased concentration causes a decrease of cell death → decreased toxicity. *(e.g. with protective compounds)*
Results isoniazid

INH, Hz and RIF increase the in vitro toxicity of isoniazid
Results pyrazinamide

- Both INH and Hz increase toxicity of PZA
Results rifampin

INH, Hz and RIF increase RIF-toxicity with no statistical conviction ($P = 0.35$ en $0.37$ with resp. INH and Hz).
In conclusion

• INH toxiciteit is increased by INH, Hz and RIF

• PZA toxicity is increased by INH and Hz

• RIF toxicity: no effects of pretreatment
Nog overwegingen

• CYP enzymen waarom: genetisch polymorfisme, protectieve interventie, gerichte focused treatment, indien predispositie kans bekend is?
In de planning, *in vitro*

- Beschermende stoffen
- Evt. selectieve remming van CYP’s: effect op toxiciteit?
- Hepatotoxiciteit HIV middelen in dit model, interacties tussen HIV en TB middelen?
- Effect van antibiotica op CYP450 activiteit bepalen (lab Farmacologie/Toxicologie NCMLS).
Recent studies on PZA toxicity 2

• Question: what is the exact role of xanthine oxidase in PZA toxicity, search for hepatoprotective compounds

Xanthine oxidase inhibition by allopurinol increases in vitro pyrazinamide-induced hepatotoxicity in HepG2 cells

Alma Tostmann$^{1,2}$, Rob E. Aarnoutse$^3$, Wilbert H.M. Peters$^4$, P.N. Richard Richard$^1$, and Martin J. Boeree$^{1,2}$

*Drug and Chemical Toxicology, 2010; 33(3): 325–328*
Pyrazinamide → Pyrazinamid deamidase → Pyrazinoic acid

Pyrazinamide → xanthine oxidase → 5-hydroxy pyrazinamide

5-hydroxy pyrazinamid deamidase → 5-hydroxy pyrazininoic acid
• Same *in vitro* model
Conclusion

• Allopurinol increases PZA toxicity \textit{in vitro}

• This is in line with increased POA induced gouty arthritis with allopurinol

• Toxicity probably caused by PZA and POA, not by the OH metabolites
Recent studies on PZA toxicity 3

- Question: are there early epigenetic markers of liver injury in PZA treatment?

Epigenetic changes in the rat livers induced by pyrazinamide treatment

V.M. Kovalenko a, T.V. Bagnyukova b, O.V. Sergienko a, L.B. Bondarenko a, G.M. Shayakhmetova a, A.V. Matvienko a, I.P. Pogribny b,*

a Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine, Kyiv, Ukraine
b National Center for Toxicological Research, Jefferson, AR, USA

Epigenetic changes

• Study in rat livers
• PZA causes epigenetic changes: changes in genes without changing the DNA sequence
  • cytosine DNA methylation decrease
  • hypomethylation of LINE 1 (long interspersed nucleotide elements)
  • aberrant promotor hypomethylation of the GSTP and $p16_{INK4A}$ genes
• Liver enzymes changed modestly at the same time
• PZA causes histological changes
After 45 days of PZA in various concentrations
250 mg PZA mg/kg/day at various time points
250 mg/PZA mg/kg/day

B: fatty infiltration of hepatocytes and non-zonal necrosis

C and D: fibrosis development
PZA and arthritis

• Assumed mechanism:

• Pyrinazoic acid is competitive with uric acid.
• PZA intake causes increase in concentration of uric acid
• When one is a susceptible person, a gouty arthritis may occur
• Allopurinol will not prevent

• Arthralgia is probably dose dependent (Pasiponodya and Gumbo, AAC, 2010)
FIG. 2. Relationship between proportions of patients receiving pyrazinamide monotherapy who developed adverse events and cumulative pyrazinamide dose. Dose-dependent toxicity was driven by arthralgia. For the two patients who had hepatotoxicity, one received 2.7 g/day and the other received 3.8 g/day of pyrazinamide.
Future research questions

• A decisive answer to the dose dependency question
• We need to know more about enzymes exactly involved in the metabolism of PZA?
• What is the exact role of interactions with other TB drugs, existing (INH, RIF) and future drugs (bedaquiline, PA 824, etc.)?
• Can we identify protective compounds?
• Pharmacogenetics, which genetic polymorphisms play a role
• What happens to PZA toxicity if we give it for longer duration (in MDRTB)
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• Richard Hafner, Dick Chaisson, Sharon Williams
Thank you
PZA punten om mee te nemen

- POA actief, PZA niet
- Begin: ik kom van HiRIF, em daarom ginteresseerd in ATDH en dus PZA
- Onze sessie heeft zelfs twee sessies niet over toxicity, zo weinig is er
- Acknowledgements
- PK?