UMC St Radboud

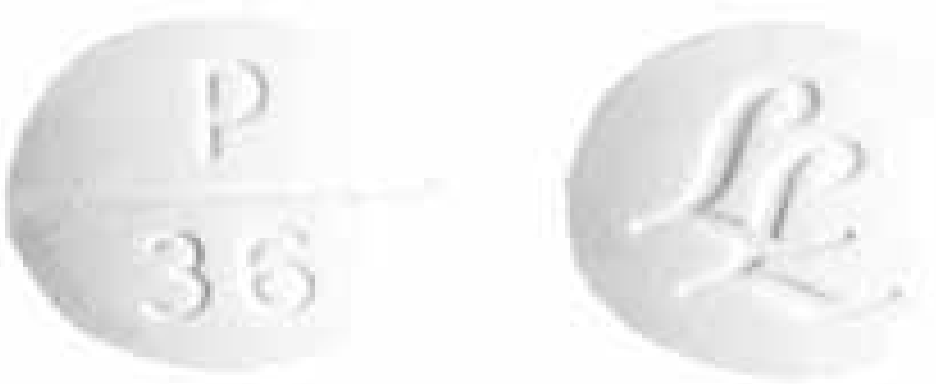
Mechanisms of pyrazinamide toxicity

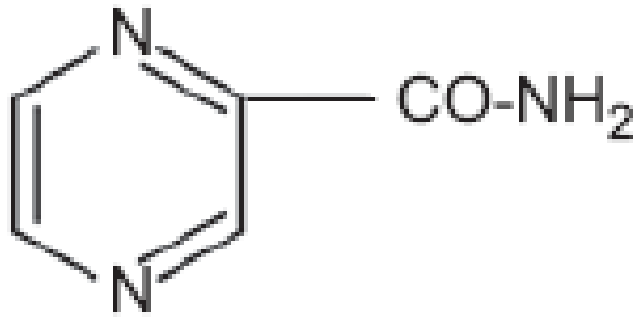
Martin Boeree, MD, PhD
Associate Professor in Medicine
Radboud University Nijmegen Medical Centre

Baltimore, September 6th, 2012

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

Pyrazinamide (pyrazinoic acid amide pyrazine carboxylamide)





Pyrazinamide

UMC St Radboud

Introduction

- Side effects profile
- Mechanisms
- Future research

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

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 ?

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

PZA toxicity

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

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

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 ?

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

[Toxic reactions during treatment with pyrazinamide **in pulmonary tuberculosis]**

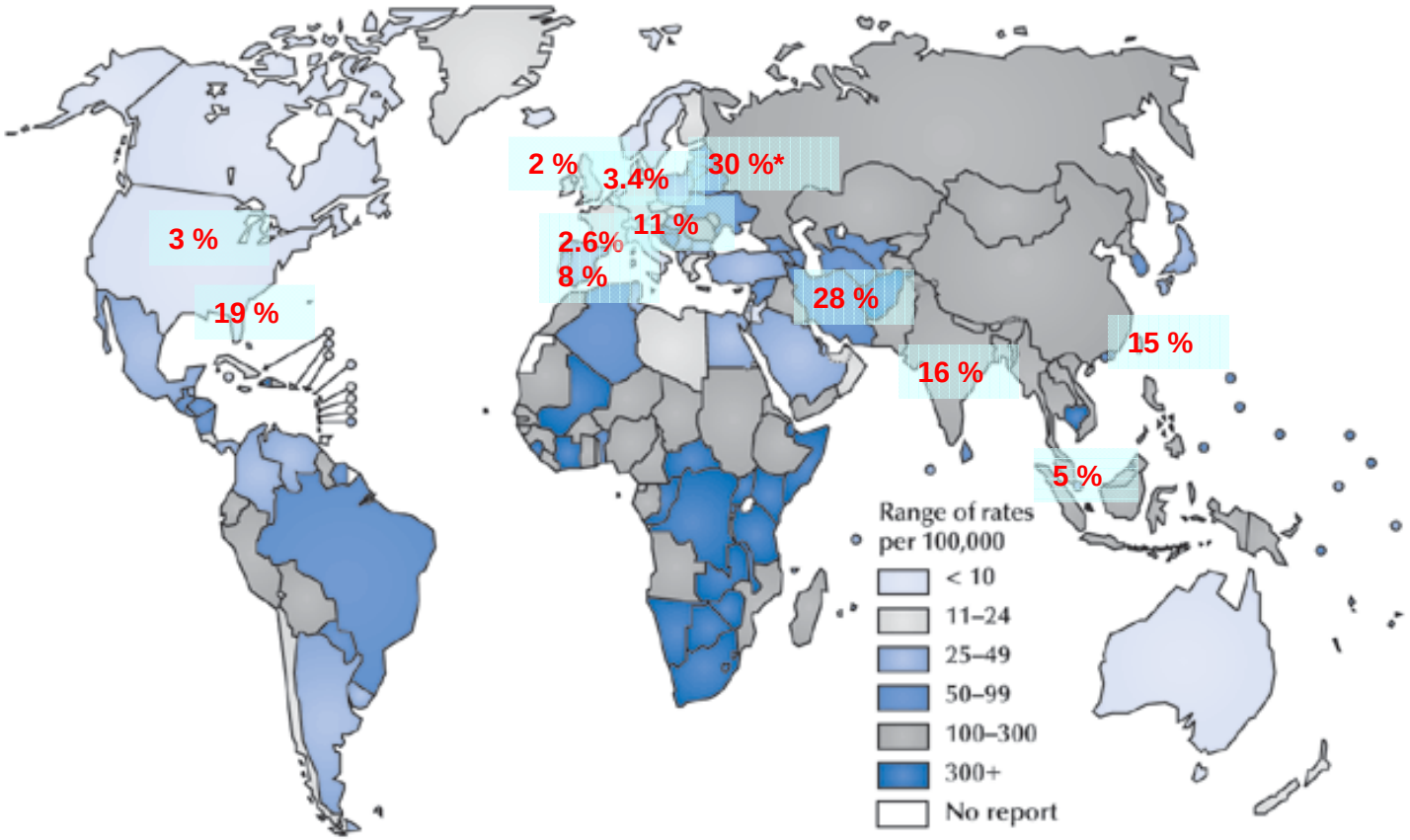
[Article in Polish]

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



UMC St Radboud

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*

*Department of Pulmonary Diseases, †University Lung Center Dekkerswald, Departments of ‡Clinical Pharmacy and §Internal Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Journal of Gastroenterology and Hepatology **23** (2008) 192–202

American Thoracic Society Documents

An Official ATS Statement: Hepatotoxicity of Antituberculosis Therapy

Jussi J. Saukkonen, David L. Cohn, Robert M. Jasmer, Steven Schenker, John A. Jereb, Charles M. Nolan, Charles A. Peloquin, Fred M. Gordin, David Nunes, Dorothy B. Strader, John Bernardo, Raman Venkataramanan, and Timothy R. Sterling, on behalf of the ATS Hepatotoxicity of Antituberculosis Therapy Subcommittee

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

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

PZA metabolism

- Metabolized and detoxified in the liver
- Excreted renally

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

Metabolism of PZA

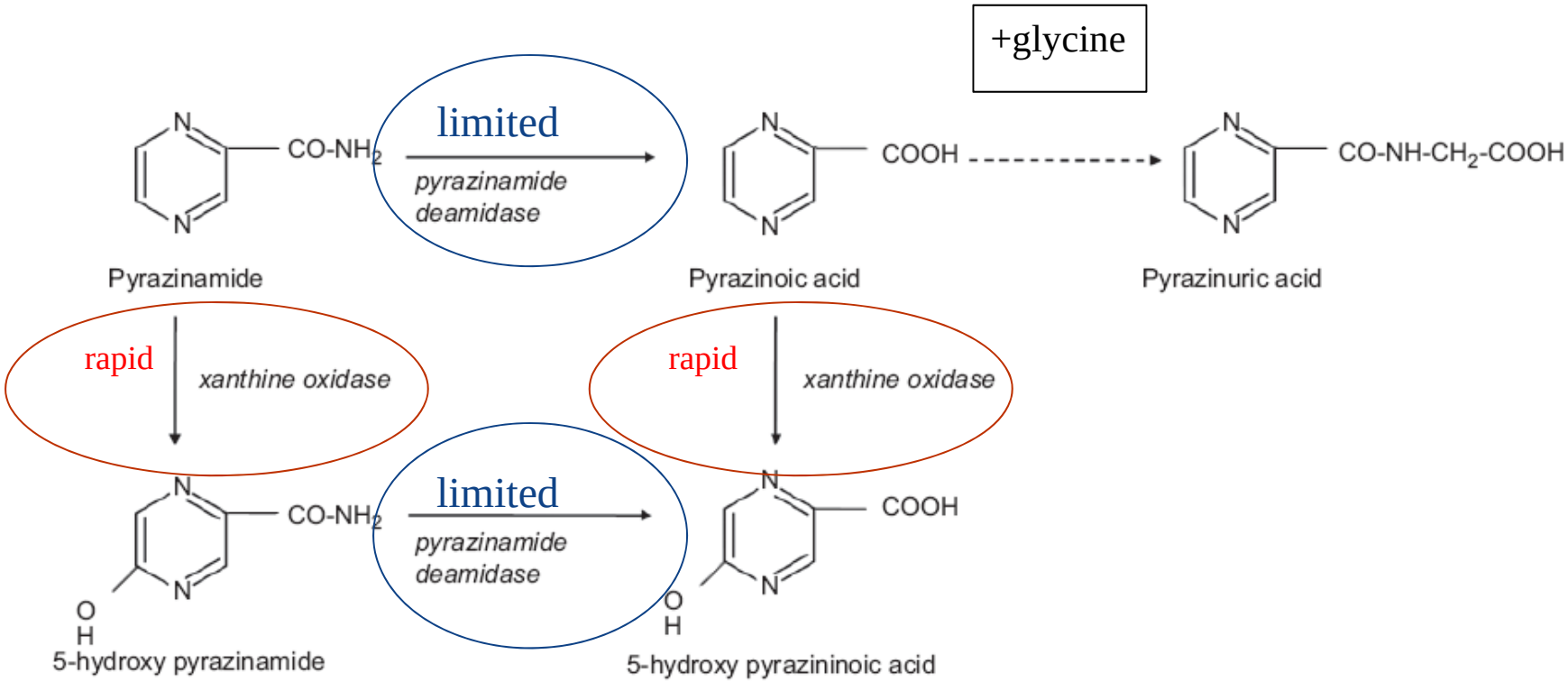


Figure 1. Metabolism of pyrazinamide (Liacroix et al., 1989).

Table 1. Pharmacokinetic parameters of PZA and its metabolites after a single oral dose of PZA 27 (3.4) mg·kg^{-1} (mean (SD))

	C_{max} (mg·l^{-1})	t_{max} (h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	MRT (h)	AUC (mg·l^{-1}·h)	CL ml·min^{-1}	V l·kg^{-1}	CL_R ml·min^{-1}	Ae 0–72 h (% dose)
PZA	38.7 (5.9)	1	1.6 (0.8)	9.6 (1.8)	11.6 (1.8)	520 (101)	61 (11)	0.71 (0.07)	1.9 (0.8)	3.2 (1.6)
PA	4.5 (0.9)	4.9 (1.7)	-	11.8 (1.6)	15.9 (3.1)	100 (21)	-	-	106 (24)	33.2 (4.5)
5-OH-PZA	1.8 (0.3)	4.0 (1.4)	-	9.7 (2.2)	13.6 (2.9)	35 (6)	-	-	197 (45)	19.7 (4.6)
5-OH-PA	0.58 (0.17)	4.2 (2.3)	-	15.3 (3.0)	17.2 (3.8)	13 (4)	-	-	464 (111)	16.5 (3.2)
PZU	-	-	-	-	-	-	-	-	-	0.15 (0.11)

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

PZA toxicity mechanism

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

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 irritaiton. May affect behavior, liver, cardiovascular system.
The toxicological properties of this substance have not been fully investigated.

Source: DrugBank

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

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

Medical Research Council Tuberculosis and Chest Diseases Unit, Brompton Hospital, Fulham Road, London SW3 6HP, England

- Maybe it is not….

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2010, p. 2847–2854 Vol. 54, No. 7
0066-4804/10/$12.00 doi:10.1128/AAC.01567-09

Clinical and Toxicodynamic Evidence that High-Dose Pyrazinamide Is Not More Hepatotoxic than the Low Doses Currently Used[▽]

Jotam G. Pasipanodya and Tawanda Gumbo*

University of Texas Southwestern Medical Center, Dallas, Texas

Received 5 November 2009/Returned for modification 28 March 2010/Accepted 22 April 2010

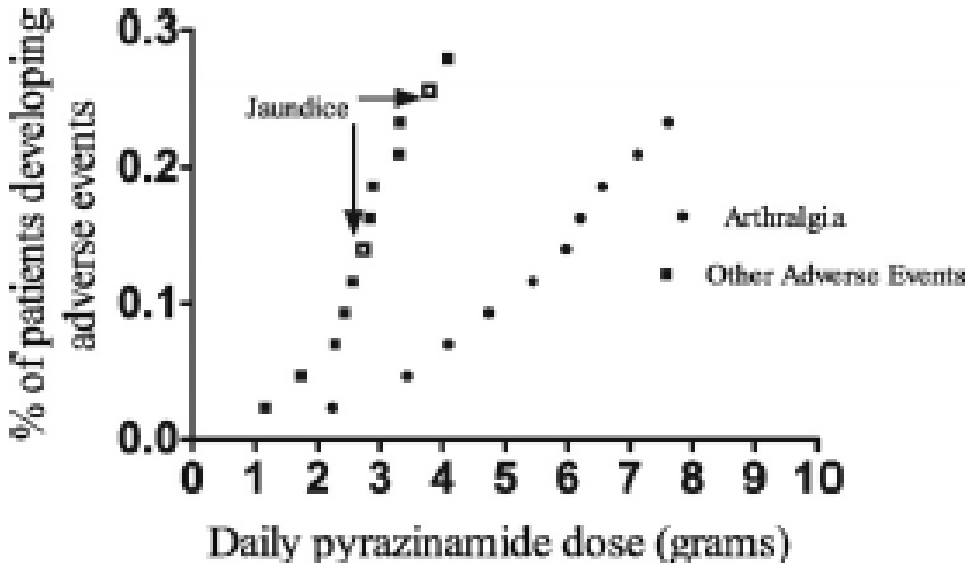


FIG. 2. Relationship between proportions of patients receiving pyrazinamide monotherapy who developed adverse events and cumulative pyrazinamide dose. Dose-dependent toxicity was driven by arthralgia. For the two patients who had hepatotoxicity, one received 2.7 g/day and the other received 3.8 g/day of pyrazinamide.

UMC St Radboud

Hepatotoxicity of Pyrazinamide

Cohort and Case-Control Analyses

Kwok C. Chang[1], Chi C. Leung[1], Wing W. Yew[2], Tat Y. Lau[1], and Cheuk M. Tam[1]

Am J Respir Crit Care Med Vol 177. pp 1391–1396, 2008

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.

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

Idiosyncratic type B drug reaction: what is it ?

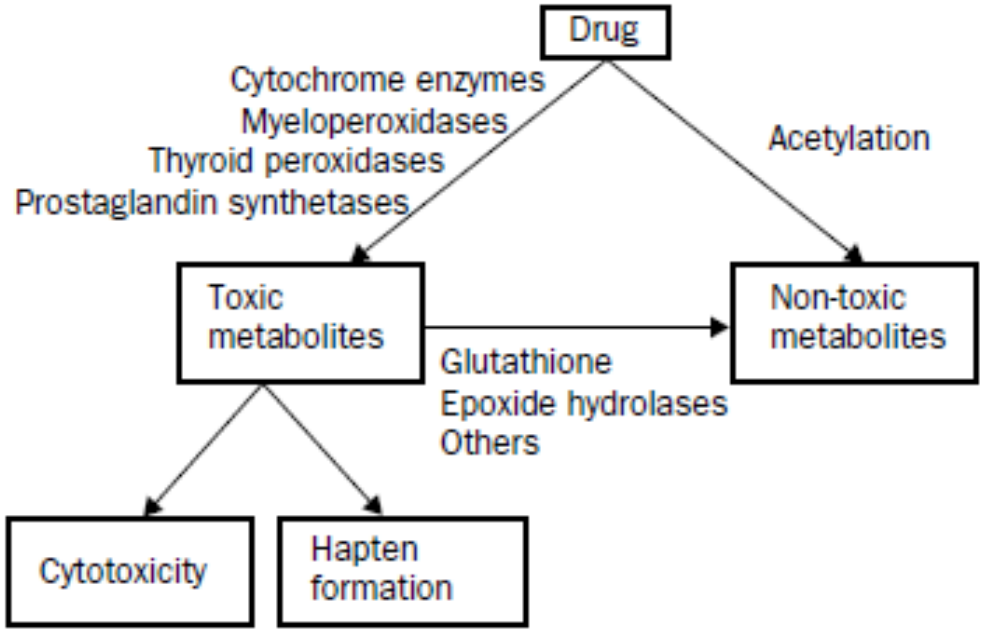
Idiosyncratic drug reactions: the reactive metabolite syndromes

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

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics



Formation and detoxification of reactive metabolites

Only idiosyncratic drug reaction
when a danger signal occurs
(cell death products)

UMC St Radboud

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]

International Journal of Antimicrobial Agents 31 (2008) 577–580

PanACEA Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

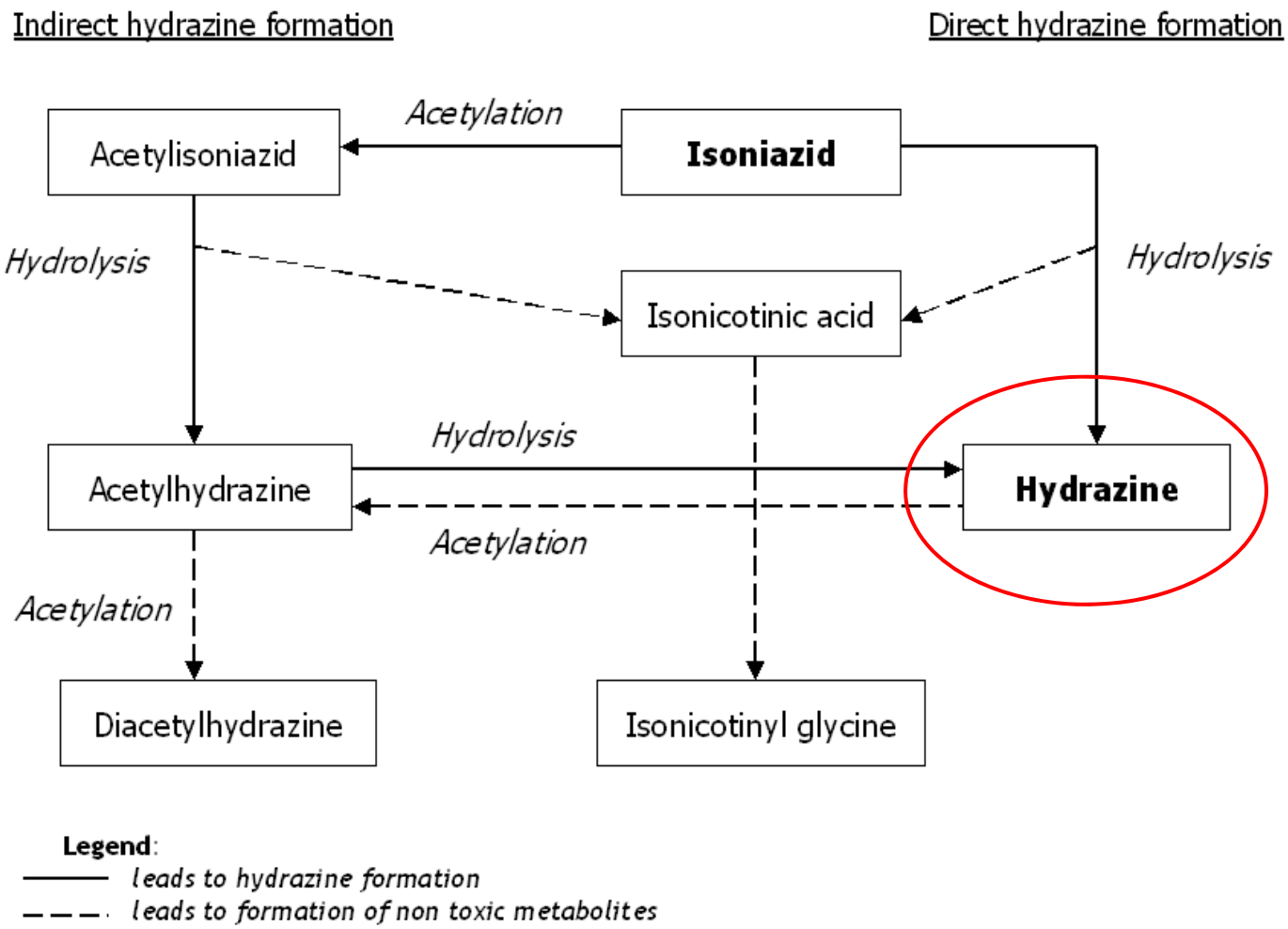
UMC St Radboud

Interactions matter

- E.g. RIF induces $CYP_{450}3A4$, therefore increases formation of hepatotoxic hydrazine
- In PZA interactions not described, and poorly investigated

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

Isoniazid metabolism



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
  - Enzyminduction with rifampin: increase of INH toxicity

INH → both induction as inhibition of CYP2E1 (dependent of concentration)

Hydrazin induces own toxicity. Repeated exposure → 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.

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

Questions *in vitro* studies

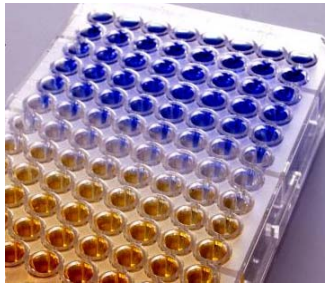
Mechanism of hepatotoxicity and interactions.

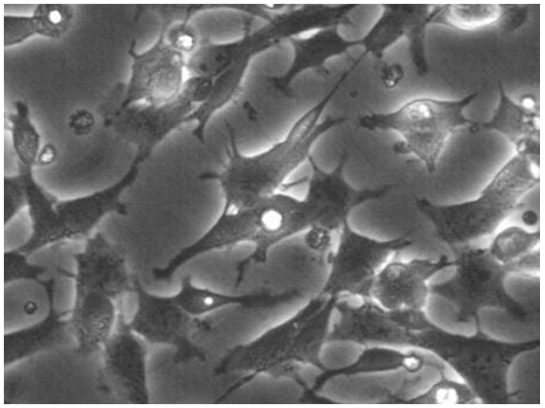
- Which 1st line TB drugs **interact** with each other in terms of toxicity
- **2RZ** (prophylactic treatment) gives severe hepatotoxicity even as compared with the standard treatment of active TB (HRZE). Can we confirm *in vitro*?

PanACEA Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

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 cellsurvival is assessed → 'toxicitycurve'.

Every dose step test 4 times repeated in triplicate

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

In vitro: WST cytotoxicity assay

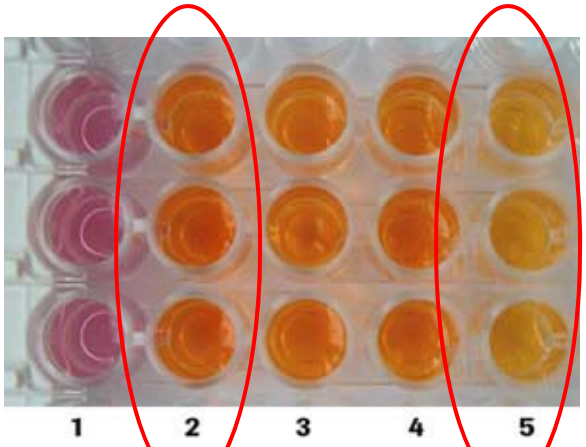
WST-protocol:

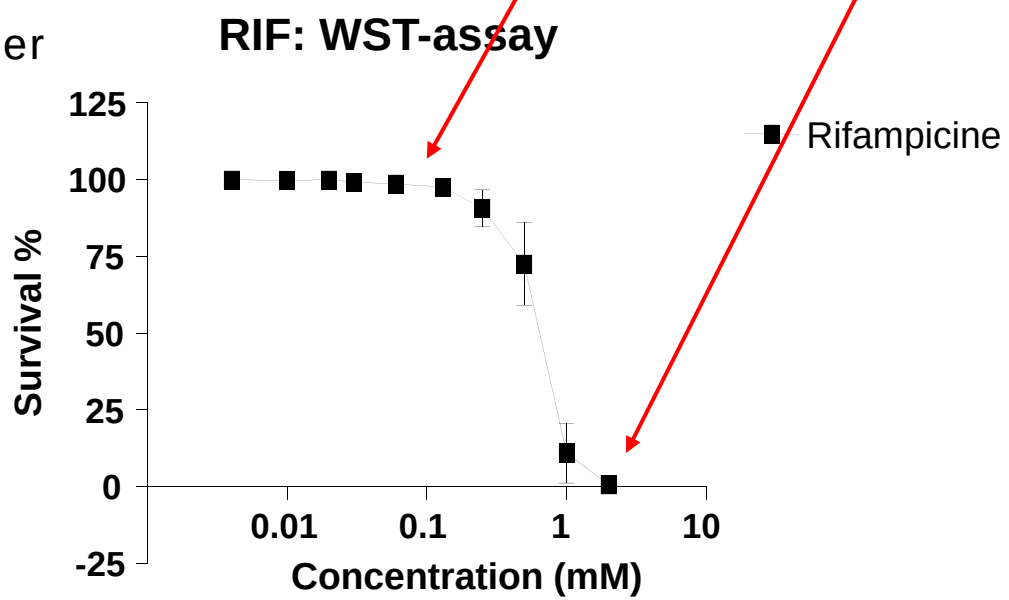
Increasing concentrations of INH, Hz, RIF, PZA

After 24 hrs: cell survival determined with WST.

WST = orange coloured which changes to dark orange by viable cells

Celll survival quantified with ELISA-reader





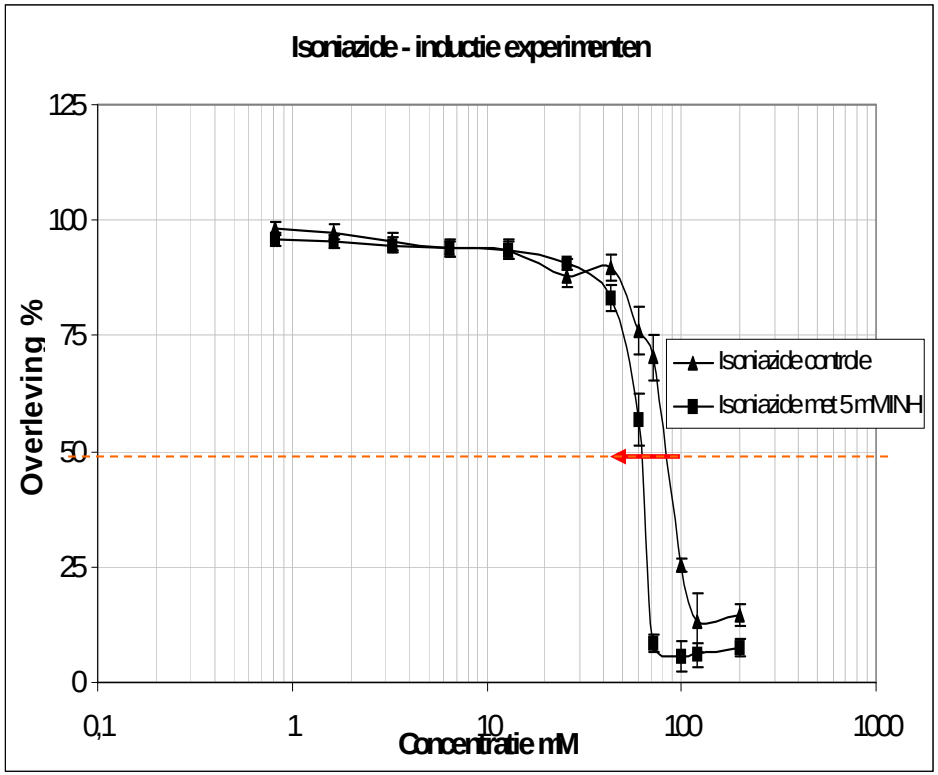
UMC St Radboud

IC50 = concentration of 50% cell death

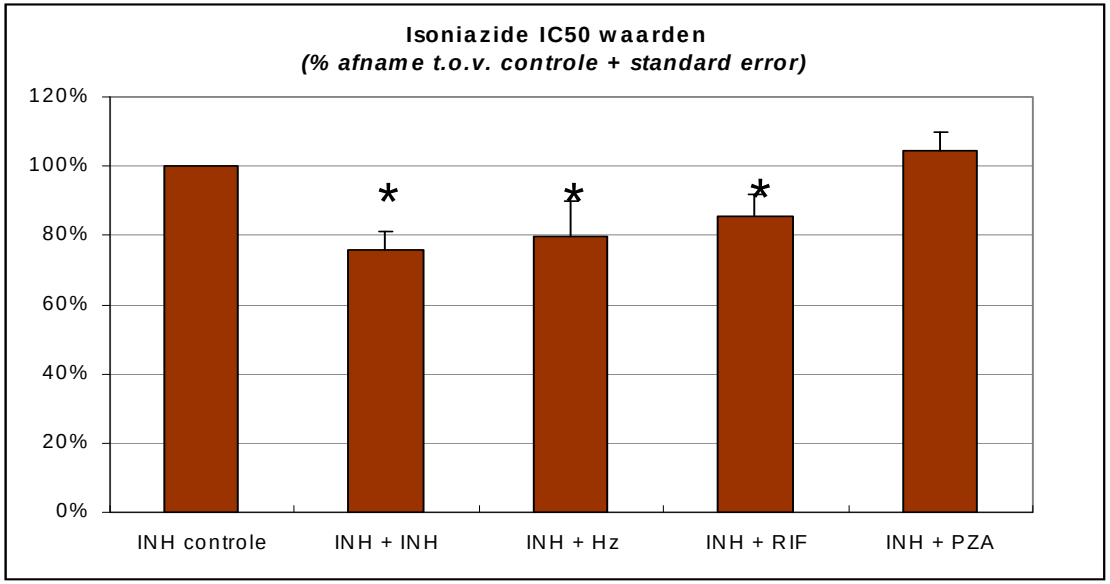
Pretreatment with other TB drugs → enzyme induction ? → effect on toxicity of TB-drugs?

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)



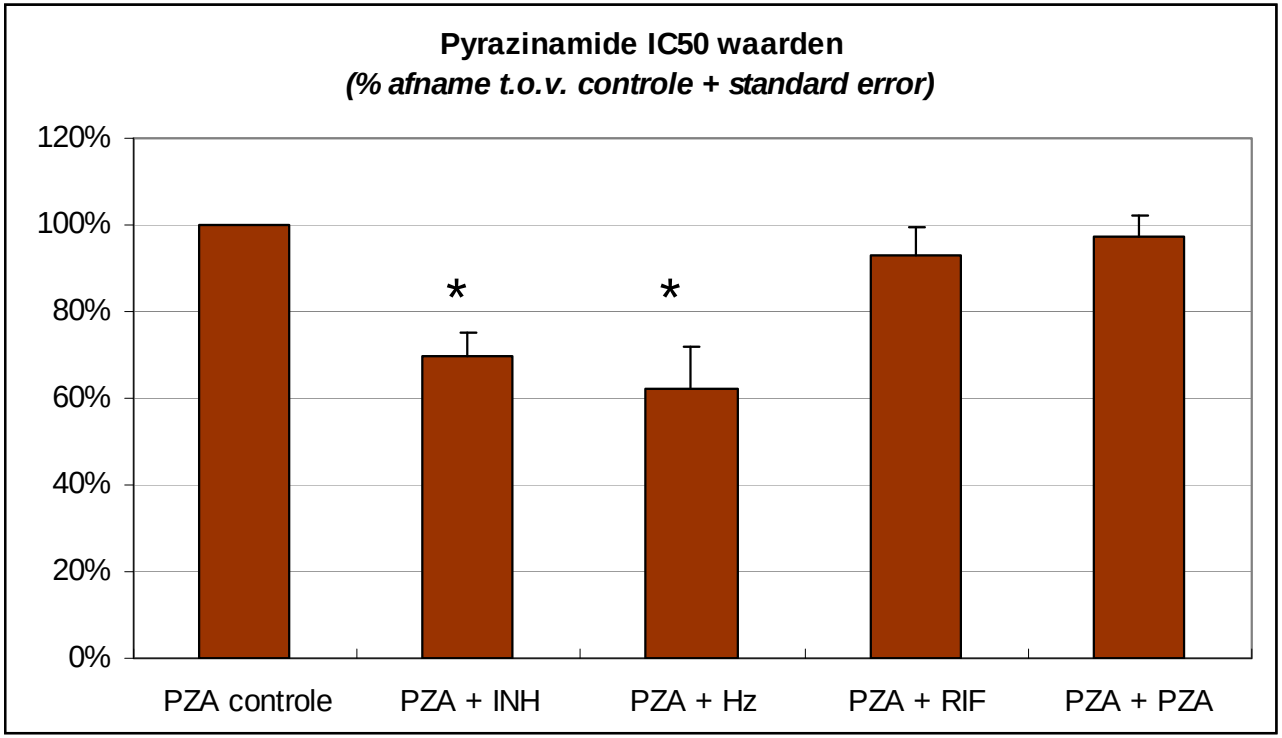
Results isoniazid



** P = 0.001, 0.076 en 0.035*

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

Results pyrazinamide

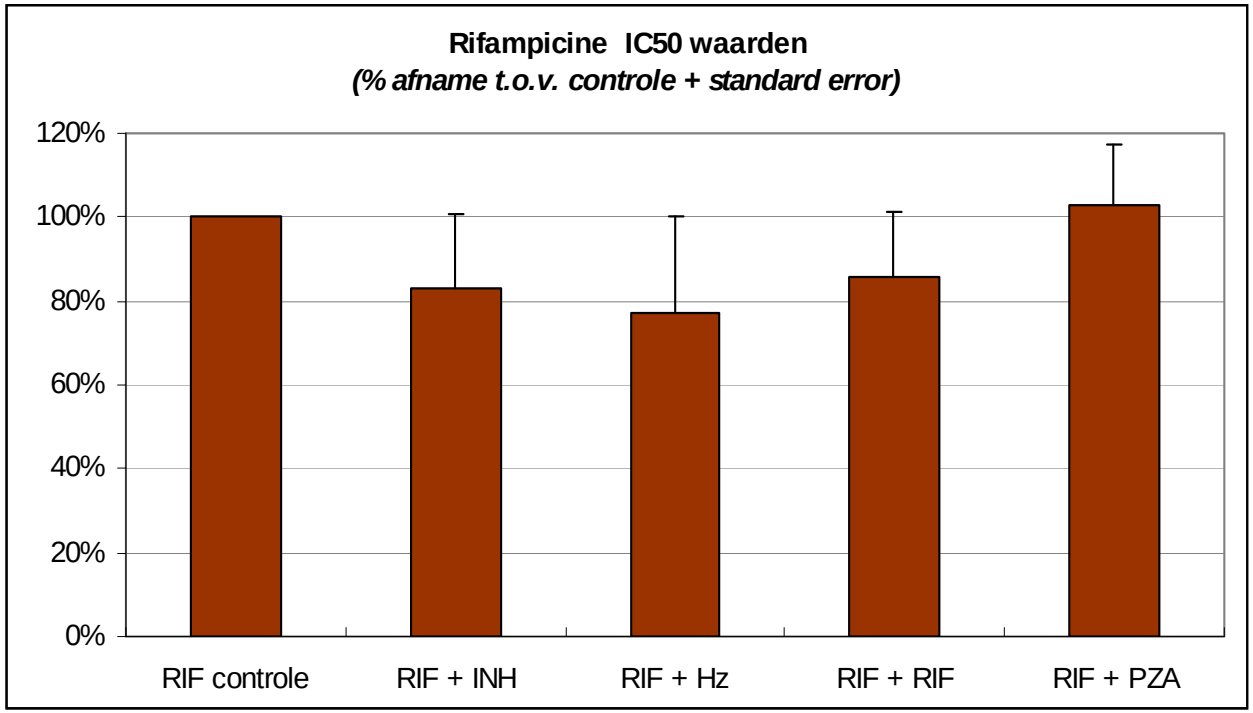


** P = 0.010 en 0.017*

- **Both INH and Hz increase toxicity of PZA**

UMC St Radboud

Results rifampin



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

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

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

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

Nog overwegingen

- CYP enzymen waarom: genetisch polymorfisme, protectieve interventie, gerichte focused treatment, indien predispositie kans bekend is?

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

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).

UMC St Radboud

Recent studies on PZA toxicity 2

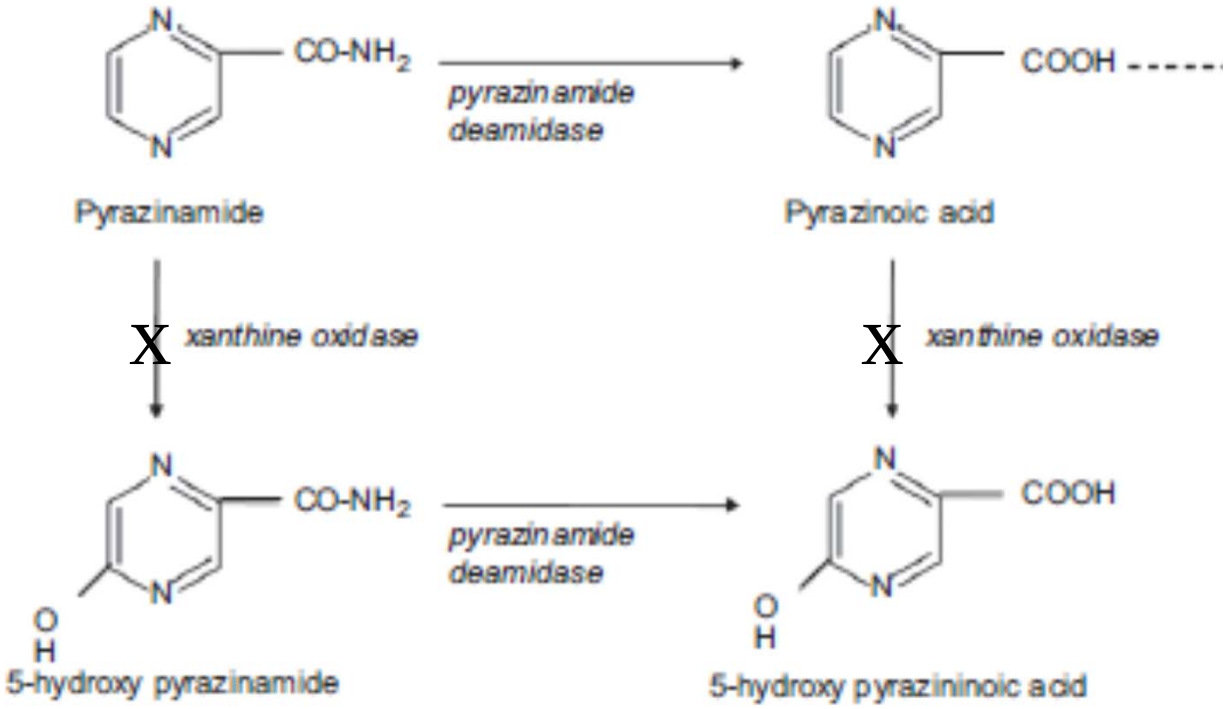
- Question: what is the exact role of xanthine oxidase in PZA toxicity, search for hepatoprotective compounds

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

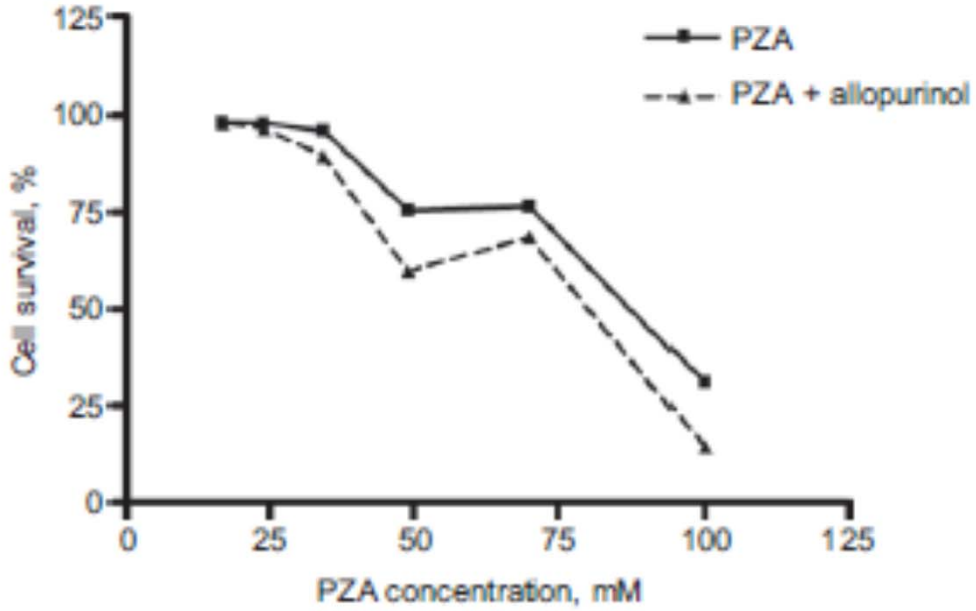
Alma Tostmann[1,2], Rob E. Aarnoutse[3], Wilbert H.M. Peters[4], P.N. Richard Richard[1], and Martin J. Boeree[1,2]

Drug and Chemical Toxicology, 2010; 33(3): 325–328

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics


CO-NH2
pyrazinamide deamidase
COOH
Pyrazinamide
Pyrazinoic acid
X
xanthine oxidase
X
xanthine oxidase
CO-NH2
pyrazinamide deamidase
COOH
OH
OH
5-hydroxy pyrazinamide
5-hydroxy pyrazininoic acid

- Same *in vitro* model



UMC St Radboud

Conclusion

- Allopurinol increases PZA toxicity *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

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

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*

Toxicology and Applied Pharmacology 225 (2007) 293–299

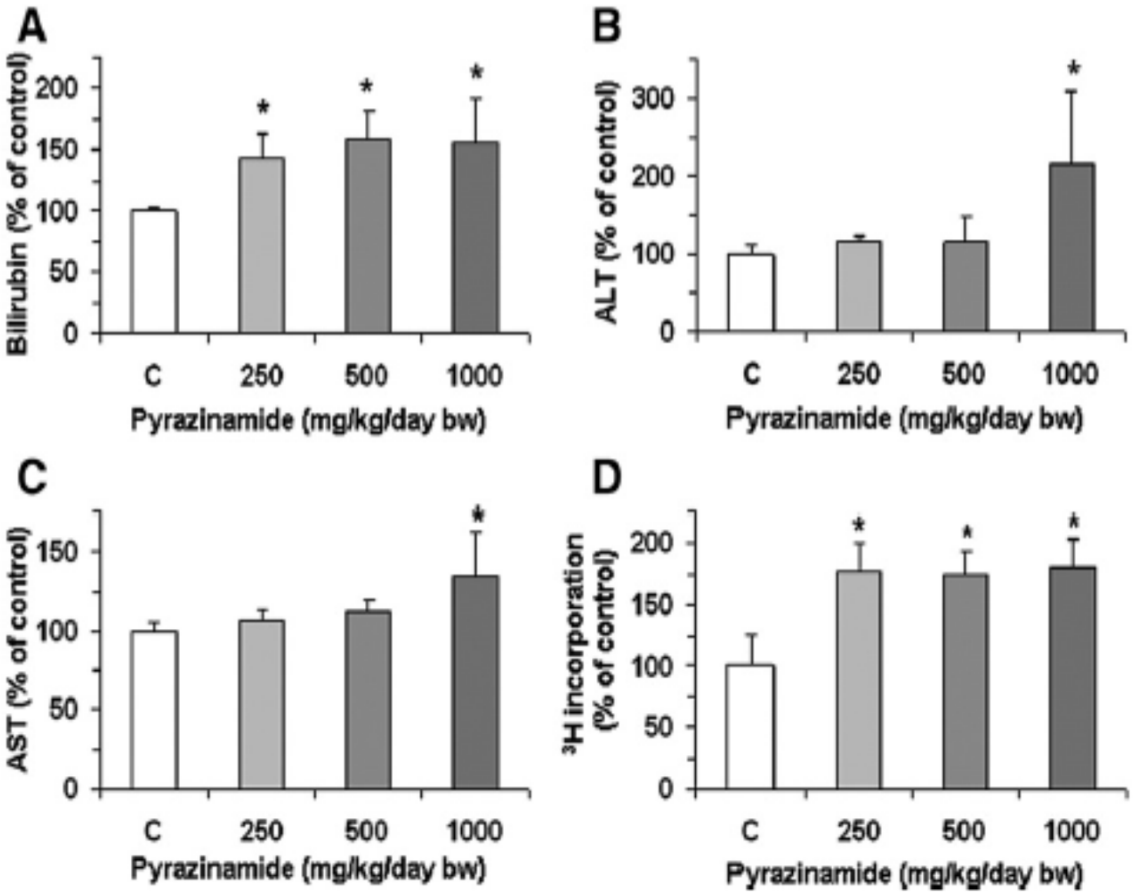
PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

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)
  - abberant promotor hypomethylation of the GSTP and *p16^{INK4A}* genes
- Liverenzymes changed modestly at the same time
- PZA causes histological changes

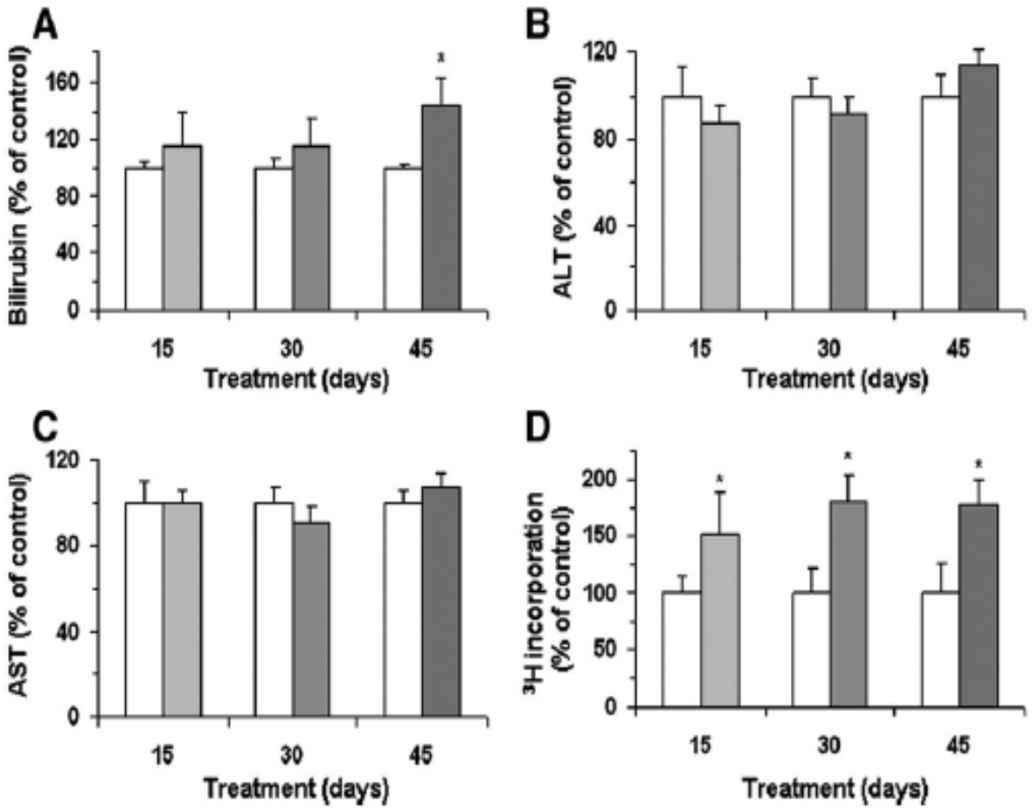
PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics



hypomethylation

After 45 days of PZA in various concentrations

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

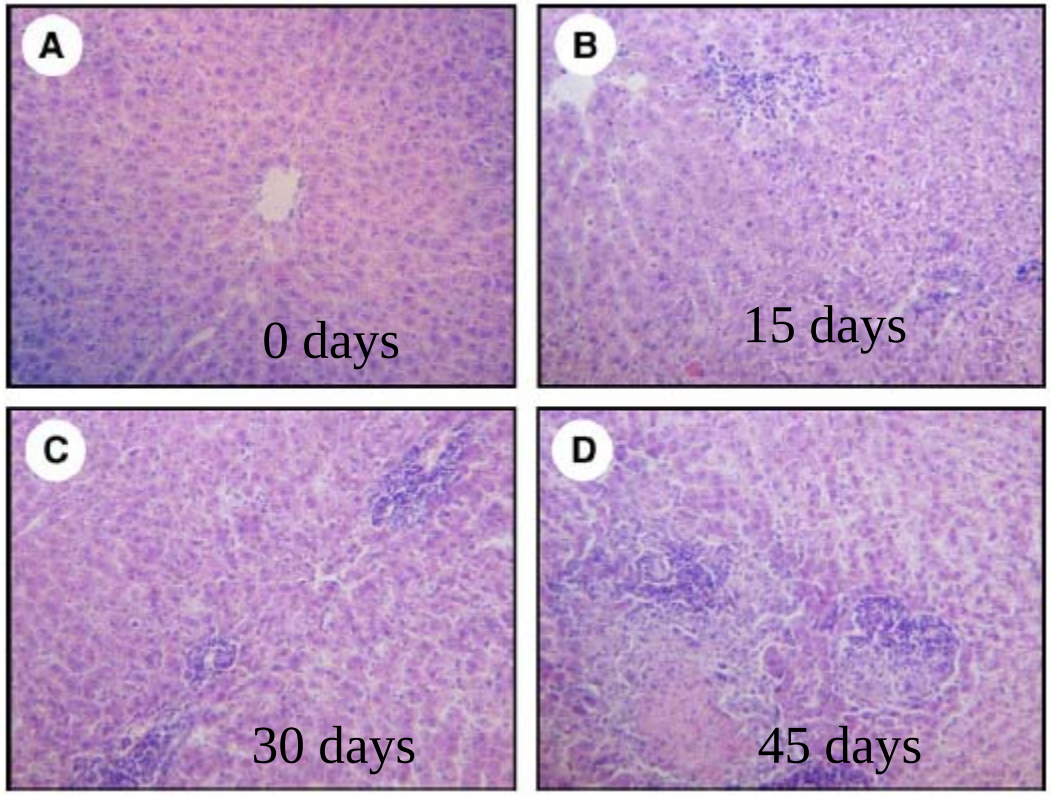


hypomethylation

250 mg PZA mg/kg/day at various time points

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

250 mg/PZA mg/kg/day



B: fatty infiltration of hepatocytes and non-zonal necrosis

C and D: fibrosis development

UMC St Radboud

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)

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

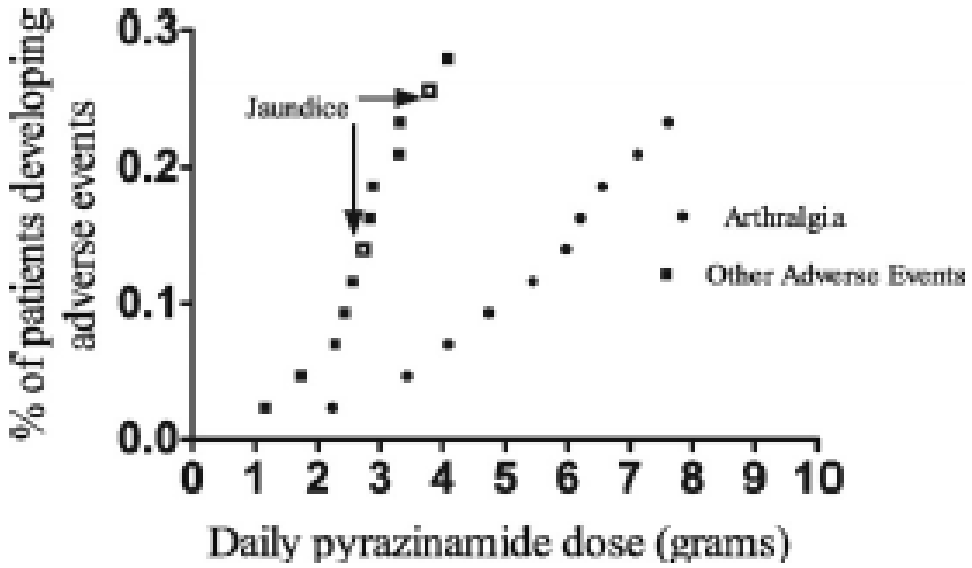


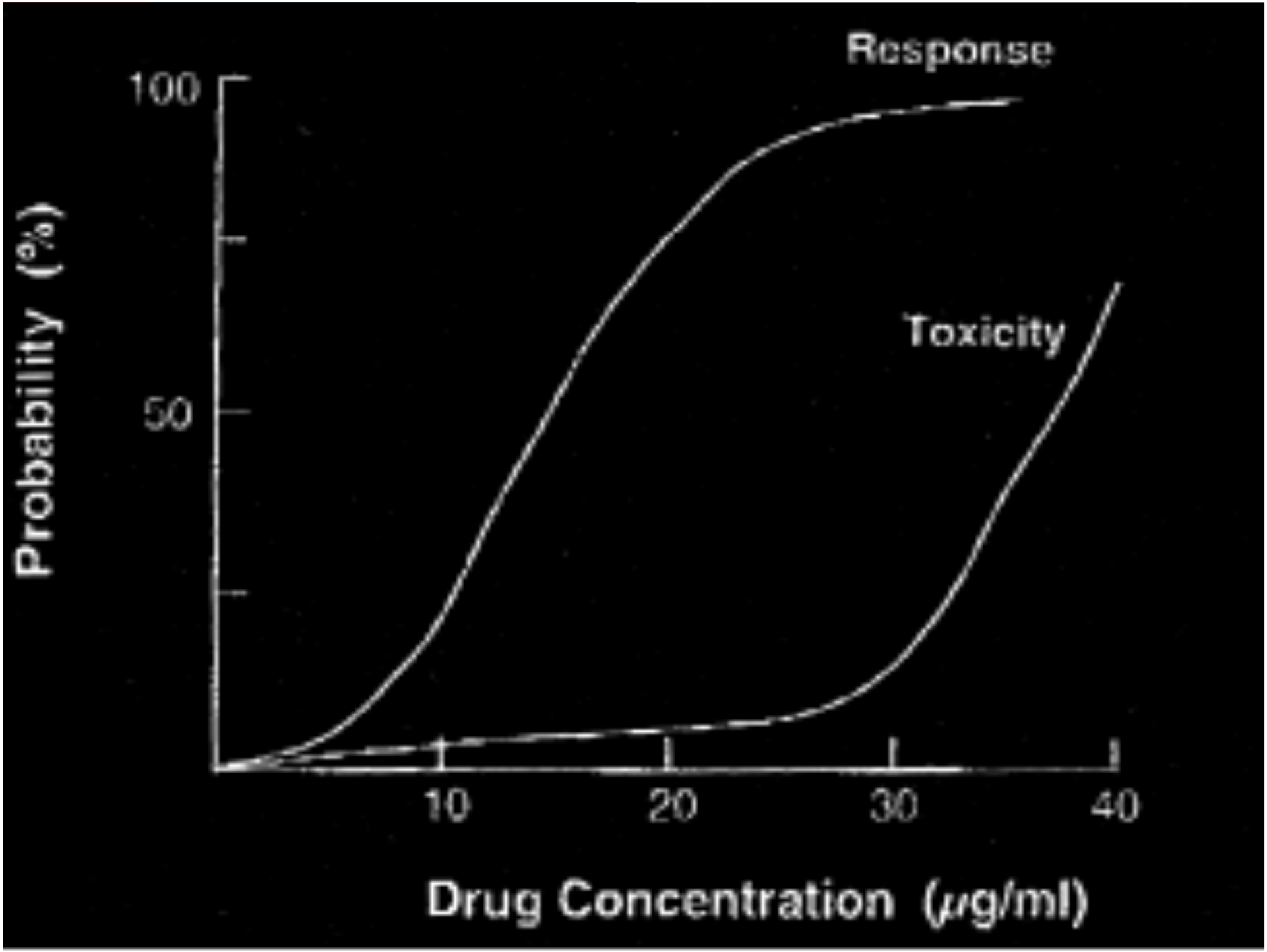
FIG. 2. Relationship between proportions of patients receiving pyrazinamide monotherapy who developed adverse events and cumulative pyrazinamide dose. Dose-dependent toxicity was driven by arthralgia. For the two patients who had hepatotoxicity, one received 2.7 g/day and the other received 3.8 g/day of pyrazinamide.

UMC St Radboud

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)

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics


Response
100
Probability (%)
50
Toxicity
10
20
30
40
Drug Concentration (μg/ml)

UMC St Radboud

Acknowledgements

- Alma Tostmann, Rob Aarnoutse, Jakko van Ingen, RUNMC
- Cecil Magis, Wouter Hoefsloot, Dekkerswald and RUNMC
- Wibert Peters, Dep of GE, RUNMC
- André van der Ven, Dep of Medicine, RUNMC
- Thanks for the organizers: NIAID, BMG Foundation, JHU Center for Aids Research, Stop TB WGND
- Richard Hafner, Dick Chaisson, Sharon Williams

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics

UMC St Radboud

Thank you

UMC St Radboud

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?

PanACEA
Pan African Consortium for the Evaluation of Antituberculosis Antibiotics