Synergistic Activation of Human Xenobiotic Receptor by Binary Cocktails of Pharmaceutical and Environmental Compounds

Perturbateurs endocriniens : à plusieurs, encore plus toxiques
≈150,000 synthetic chemicals used in consumer products

Dissemination into the environment

Adverse effects on the endocrine system
- endocrine disrupting chemicals (EDCs)
- interaction with nuclear receptors
Nuclear hormone receptors
Biological function

Simplified view of hormone action through nuclear receptors
A highly controlled process through fine spatio-temporal tuning of hormonal signal
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Misregulation of the nuclear hormone receptor activity =
cancers, infertility, diabetes, obesity, ...
The cocktail effect
Additive, antagonistic or synergistic?
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Individuals are chronically exposed to cocktail of exogenous substances that may have harmful effects
The cocktail effect

Our target: PXR, the xenobiotic sensor

PXR = Pregnane X Receptor, or Steroid X Receptor
Up-regulates major detoxification genes (redox reactions, conjugation, excretion)

Xenobiotics (drugs, pesticides, chemicals, …)
The cocktail effect

*Our target: PXR, the xenobiotic sensor*

PXR = Pregnane X Receptor, or Steroid X Receptor
Up-regulates major detoxification genes (redox reactions, conjugation, excretion)

- **PXR has a large ligand binding pocket**
- **Hyper-activation of PXR**
  - Drug-drug interactions
  - Deregulation of natural hormones homeostasis
  - Chemo-resistance (+++)
  - Cancer aggressiveness (+++)
The cocktail effect

Strategy, 1 = screening and in vitro studies

Medium-throughput screening on HG5LN-PXR reporter cell line

Delfosse et al., 2015
Nature Communications
The cocktail effect
*Strategy, 1 = screening and in vitro studies*

Medium-throughput screening on HG5LN-PXR reporter cell line

First screen with 40 molecules (drugs, pesticides, industrial products, etc...) = 780 binary mixtures

Together, these compounds produced more than an additive effect

Delfosse et al., 2015
*Nature Communications*
The cocktail effect

Strategy, 1 = screening and in vitro studies

Synergistic activation of PXR by EE2 and TNC in different human cell lines

Hepatocellular carcinoma

Colon adenocarcinoma

HepG2

Theoretical additivity curve

Delfosse et al., 2015, Nature Communications
The cocktail effect

**Strategy, 1 = screening and in vitro studies**

Cocktail effect on CYP3A4 expression and activity

**LS174T**

Colon adenocarcinoma

**Primary human hepatocytes (PHHs)**

Liver samples from adult patients

Freshly isolated PHHs

Delfosse et al., 2015, Nature Communications
The cocktail effect

Strategy, 2 = biophysical studies

Mass spectrometry under native conditions

No ligand added

PXR LBD
Theoretical mass = 38 217.7 Da

Delfosse et al., 2015
Nature Communications
The cocktail effect

Strategy, 2 = biophysical studies

Mass spectrometry under native conditions

PXR LBD
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EE2
(296.4 g.mol\(^{-1}\))

TNC
(444.2 g.mol\(^{-1}\))

Delfosse et al., 2015
Nature Communications
The cocktail effect
Strategy, 2 = biophysical studies

Mass spectrometry under native conditions

PXR LBD
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No ligand added

EE2
(296.4 g.mol\(^{-1}\))

TNC
(444.2 g.mol\(^{-1}\))

EE2
+ 297 Da

TNC
+ 445 Da

TNC
+ 740 Da

Delfosse et al., 2015
Nature Communications
The cocktail effect

*Strategy, 2 = biophysical studies*

**EE2 affinity**

- **PXR/EE2**
  - $K_d = 10.80 \pm 4.00 \mu M$

**TNC affinity**

- **PXR/TNC**
  - $K_d = 14.30 \pm 1.41 \mu M$

**PXR+TNC/EE2**

- $K_d = 173.25 \pm 61.16 \text{ nM}$

**PXR+EE2/TNC**

- $K_d = 328.17 \pm 91.36 \text{ nM}$

Both sets of data are scaled by factors of 60 and 40, respectively.
The cocktail effect

Strategy, 2 = biophysical studies

EE2 and TNC bind cooperatively to PXR

The binary cocktail has a nanomolar affinity

Delfosse et al., 2015, Nature Communications
The cocktail effect

Strategy, 3 = structural studies

Fo-Fc omit maps (3σ)

EE2

TNC

PDB id = 4X1G
res = 2.25 Å
R / R_free = 0.174 / 0.218

Delfosse et al., 2015, Nature Communications
The cocktail effect

Strategy, 3 = structural studies
The cocktail effect

**Strategy, 3 = structural studies**
The cocktail effect

**Strategy, 3 = structural studies**

*Supramolecular ligand*
The cocktail effect
*Proof of concept*

1st screening with 40 molecules (receptor = PXR)
- ethinylestradiol (EE2, contraceptive)
- transnonachlor (TNC, pesticide)

Synergy observed in human cell lines
Transactivation
Hepatocellular carcinoma

Mutual stabilisation in the LBP
res = 2.25 Å

Cooperative binding (ITC)
(EE2 → TNC+EE2)

HepG2

Transactivation

K_{d} = 10.8 \mu M

K_{d} = 0.17 \mu M
Conclusion

The association makes the poison

Paracelsus (1493-1541)

The dose makes the poison
All substances are poisonous
There is none which is not a poison
The right dose differentiate a poison and a remedy

... The association makes the poison
Conclusion

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Supramolecular ligand: molecular assembly consisting of two or more compounds that interact with each other inside the LBP of a receptor, resulting in the creation of a new entity with improved functional characteristics in regard to those of its individual components.
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Huge chemical/size diversity of xenobiotics + high structural plasticity of LBPs, the mechanism defined for PXR is likely to occur also with other NRs.
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Broad reaching implications for the fields of endocrine disruption, toxicology, and chemical risk assessment.
The cocktail effect

*Integrative approach*

**Identify synergistic cocktails**
- HT screening in luciferase reporter system
  - *Platform PCC – IRCM*

  - *Environnamental compounds (200)*
  - Drugs (1280)
  - Natural products (320)

**Assess the degree of synergy in vitro**

- *Native MS*
  - Fluo. aniso.

**Elucidate the mechanism of action of cocktails at atomic level**

**In situ and in vivo validations**

- *Human cell lines, tissues, xenografts*
  - *P. Balaguer (IRCM, Mtplr)*
  - *J.-M. Pascussi (IGH, Mtplr)*

- *Larval systems (xenopus and zebrafish)*
  - *V. Laudet (OOB, Banyuls)*
  - *B. Demeinex (MNHM, Paris)*
CBS (Montpellier)  
Structural and biophysical analyses

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Screening & functional studies

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Abdelhay Boulah Touf  
Patrick Balaguer

IGF (Montpellier)  
Functional studies

Bertrand Beucher  
Jean-Marc Pascussi
The cocktail effect
Variations on a theme

17α-ethinylestradiol (EE2)  cis-chlordane (CC)

17β-estradiol (E2)  trans-nonachlor (TNC)

--- Theoretical additivity curve

Delfosse et al., 2015, Nature Communications