PPAR α ligand protect the cisplatin-induced nephrotoxicity

Renu Bhatt, Ph.D
University of Arkansas for Medical Science, Little Rock, Arkansas
PPAR α ligands - Wy-14,643
& bezafibrate
PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS

• PPAR-α is a nuclear protein.
• Highly expressed in:

• These areas are considered metabolically very active.
• Administration of PPAR-α ligands leads to the activation of PPAR-α.
• This results in a pleiotropic response that includes:
  • Increased peroxisomes proliferation
  • Upregulation of fatty acid oxidation
  • Reduced inflammation
  • Suppression of apoptosis.
PLATINUM (II) COMPOUNDS

Cisplatin

Cl

Pt

NH₃

Cl

NH₃

Cl

CDEP

H₂N

Pt

H₂N

H₂N
• Studies from Dr. Portilla’s lab and others demonstrated decreased expression of PPAR-\(\alpha\) in response to cisplatin nephrotoxicity.

• This resulted in reduced enzyme activity of several kidney PPAR-\(\alpha\) target genes.

• The use of PPAR-\(\alpha\) ligands protected renal function.

• Protective function via prevention of proximal tubule cell death
Ischemia/Reperfusion, Nephrotoxins

Bad, Bid, Bim, Hrk, Puma, Noxa, etc.

Bad, Bid, Bim, Hrk, Puma, Noxa, etc.

Bcl-2, Bcl-x<sub>L</sub>

Bax, Bak, etc.

Mitochondria

Smac

Cytochrome c

AIF

EndoG

Loss of Mitochondrial Function

IAP

Caspase-9

Caspase-3

Apoptosis
OBJECTIVE

Examine the mechanisms by which PPAR-α ligand protects renal function in the model of cisplatin–induced acute renal failure (ARF):

What is the role of endonuclease G (EndoG) in this pathway?
ARF induced in mice by a single i.p. dose of cisplatin (20mg/kg).
Pelleted mouse chow was prepared containing 0.1% wy-14,643.
Mice were fed the WY for seven days prior to cisplatin injection.
Mice were sacrificed and kidney tissue was frozen in liquid N₂ for RNA isolation.
Blood urea nitrogen and creatinine in serum
For kidneys were collected in 10% neutral buffered formalin for histopathology evaluation (immunohistochemistry)
Endonuclease activity assays
In situ hybridization
TUNEL assay
Western blot Analysis
EFFECT OF WY TREATMENT ON BLOOD UREA NITROGEN & SERUM CREATININE

A

Blood Urea Nitrogen mg/dl

Control  Cisplatin  Cisplatin+WY  WY

B

Serum Creatine mg/dl

Control  Cisplatin  Cisplatin+WY  WY
HISTOPATHOLOGICAL ALTERATION IN PPAR\(_{\alpha}\) WILD-TYPE MICE
Examine the potential mechanism by which PPAR alpha ligand ameliorates cisplatin-induced ARF:
EFFECT OF WY TREATMENT ON EndoG mRNA LEVELS
TIME COURSE: EFFECT OF CISPLATIN ON RELATIVE EndoG LEVELS

By day 4 of cisplatin, EndoG protein is increased nearly eight-fold.

PPARα +/-

Control D1 D2 D3 D4

Cisplatin

Relative amount of EndoG Protein

* * *
EFFECT OF WY TREATMENT ON EndoG LEVELS

PPARα +/-

Control Cisplatin WY WY + Cisplatin

EndoG

1 2 3 4

Control Cisplatin WY WY + Cisplatin
EFFECT OF WY TREATMENT ON EndoG LEVELS

This rise in EndoG was not blocked by WY in PPAR-α null mice.
Our finding suggests that the protective effect of PPAR-α ligands depends on an intact and functionally active PPAR α gene.
IN SITU HYBRIDIZATION – LOCALIZATION OF EndoG
IMMUNOLOCALIZATION OF EndoG

(A) [Image of tissue section labeled PT with arrows indicating areas of interest]

(B) [Image of tissue section labeled PT with arrows indicating areas of interest]

(C) [Image of tissue section labeled PT with arrows indicating areas of interest]

(D) [Image of tissue section labeled PT with arrows indicating areas of interest]
IN SITU EVALUATION OF KIDNEY APOPTOSIS

Terminal deoxynucleotidyl transferase mediated dUTP nick-end-labeling
TUNNEL-POSITIVE NUCLEI IN KIDNEY CORTEX
EFFECT OF KIDNEY MITOCHONDRIAL LYSATE ON SUPERCOILED DNA

Lane 1 = 100%
Lane 2 & 3 = 64 & 89%
Lane 4 & 5 = 67 and 67%

Endonucleolytic nicking of supercoiled plasmid DNA
PPARα

9cis RA

Gene expression

AGGACANAGGTCA

acyl CoA oxidase
bis functional enzyme
cytochrome p4504A
apo-lipoproteins

Lipid metabolism
Peroxisome proliferation

DNA replication

Apoptosis
Section II

Fibrates prevents cisplatin-induced proximal tubule cell death: Effect of Bezafibrate
Examined the cellular mechanism by which bezafibrate prevents cisplatin induced proximal tubule cell death
TIME COURSE EFFECT OF CISPLATIN ON PRO- AND ANTI-APOPTOTIC MOLECULES

A
Mitochondria

B
Cytosol

Time (hrs)  0  3  6  12  18

Bax
Cox-IV

Time (hrs)  0  3  6  12  18

Bax
β-Actin
TIME COURSE OF Bax CONCENTRATION AND LOCALIZATION WITHIN THE CELL

- Cytosolic Bax decreases sharply while mitochondrial Bax increases roughly six-fold.
EFFECT OF BEZAFIBRATE ON ORO STAINING IN RENAL PROXIMAL TUBULE CELLS

TIME COURSE OF CISPLATIN IN NEFA IN RENAL PROXIMAL TUBULE CELLS
EFFECTS OF BEZAFIBRATE ON Bax AND Bcl-2 LOCALIZATION WITHIN THE CELL AFTER CISPLATIN

- Bezafibrate treatment prevents Bax migration to the mitochondria.
- This may be due to an increase in cytosolic Bcl-2.
Bezafibrate treatment prevents Bax migration to the mitochondria.

This may be due to an increase in cytosolic Bcl-2.
Time course of cisplatin induced cytochrome C release

A. Cytosol
- Time in hours: 0, 1, 3, 6, 9, 12
- Cytochrome C
- β-Actin

B. Mitochondria
- Time in hours: 0, 1, 3, 6, 9, 12
- Cytochrome C
- Cox-IV

C. Cytosol
- Time in hours: 0, 1, 3, 6, 9, 12
- Cytochrome C
- β-Actin

D. Mitochondria
- Time in hours: 0, 1, 3, 6, 9, 12
- Cytochrome C
- Cox-IV

Drug treatment:
- Bezafibrate: - - + + + +
- Cisplatin: - + - - + +
30 kDa

17kDa

Pro-Caspase-3

Active-Caspase3

β-Actin

Relative amount of Active caspase3

Cisplatin

-  +  -  +

0  1  2  3  4  5
Ischemia/Reperfusion, Nephrotoxins

Bad, Bid, Bim, Hrk, Puma, Noxa, etc.

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Bax, Bak, etc.

Mitochondria

Smac

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EndoG

? ±

Loss of Mitochondrial Function

IAP

Caspase-9

Caspase-3

Apoptosis

Apaf-1

Caspase-9

Apoptosis
CONCLUSIONS

• Our finding suggest that increased Endo-G expression facilitates DNA fragmentation and apoptotic cell death in renal proximal tubule after cisplatin treatment.
• Pre-treatment with PPAR-α ligands (Wy) protects kidney function.
• Bezafibrate prevented apoptotic cell death at various levels:
  • (1) It increased the expression of antiapoptotic Bcl-2
  • (2) It prevented the inhibition of PPAR-α activity and the accumulation of non esterified free fatty acid
  • (4) It prevented the release of cytochrome c from the mitochondria to the cytosolic compartment
• (5) bezafibrate prevented cisplatin induced caspase-3 activation
• All these intracellular events resulted in amelioration of apoptotic cell death
Thank You
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