

PPAR α ligand protect the cisplatin -induced nephrotoxicity

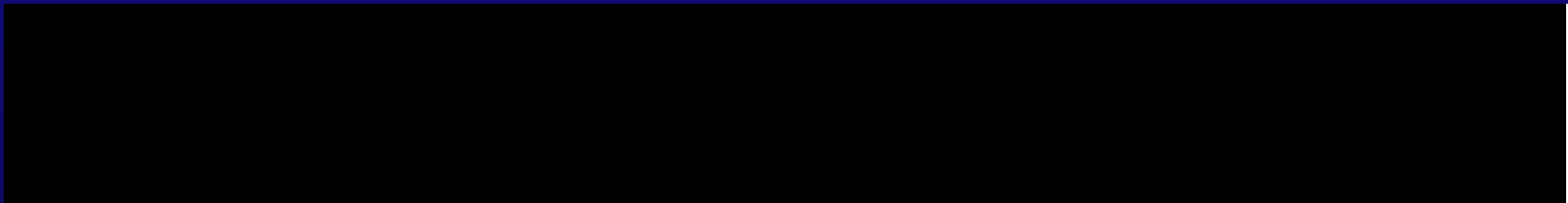
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University of Arkansas for Medical Science, Little Rock, Arkansas

**PPAR α ligands - Wy-14,643
& bezafibrate**

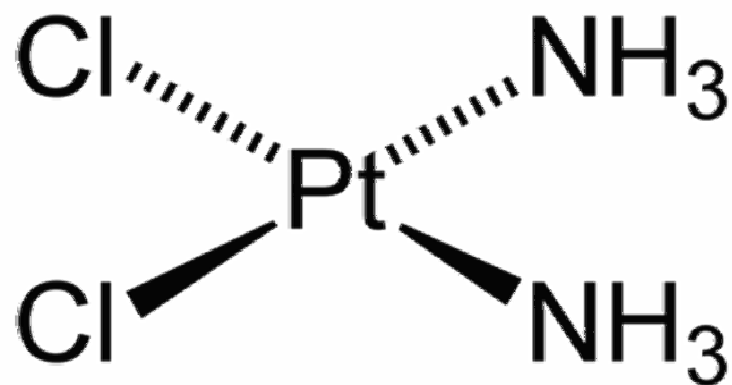
PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS

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

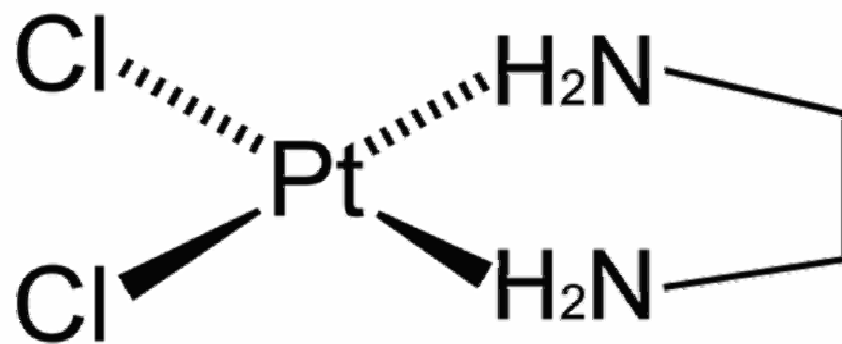


- **These areas are considered metabolically very active.**
- **Administration of PPAR- α ligands leads to the activation of PPAR- α .**
- **This results in a pleiotropic response that includes:**
 - **Increased peroxisomes proliferation**
 - **Upregulation of fatty acid oxidation**
 - **Reduced inflammation**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

PLATINUM (II) COMPOUNDS



Cisplatin



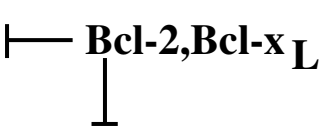
CDEP

- **Studies from Dr. Portilla's lab and others demonstrated decreased expression of PPAR- α in response to cisplatin nephrotoxicity.**
- **This resulted in reduced enzyme activity of several kidney PPAR- α target genes.**
- **The use of PPAR- α ligands protected renal function.**
- **Protective function via prevention of proximal tubule cell death**

Ischemia/Reperfusion, Nephrotoxins



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

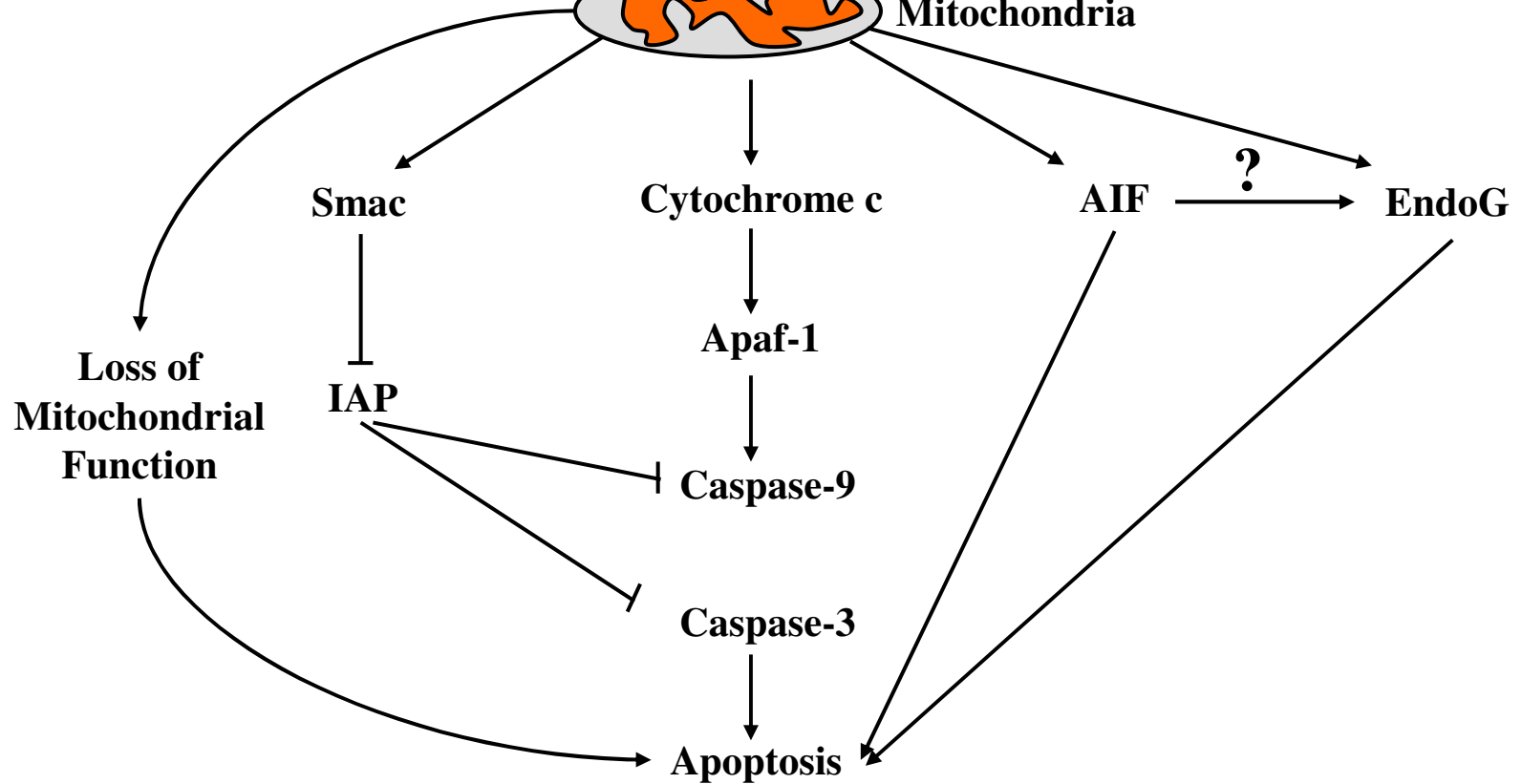


Bcl-2, Bcl-x_L

Bax, Bak, etc.



Mitochondria



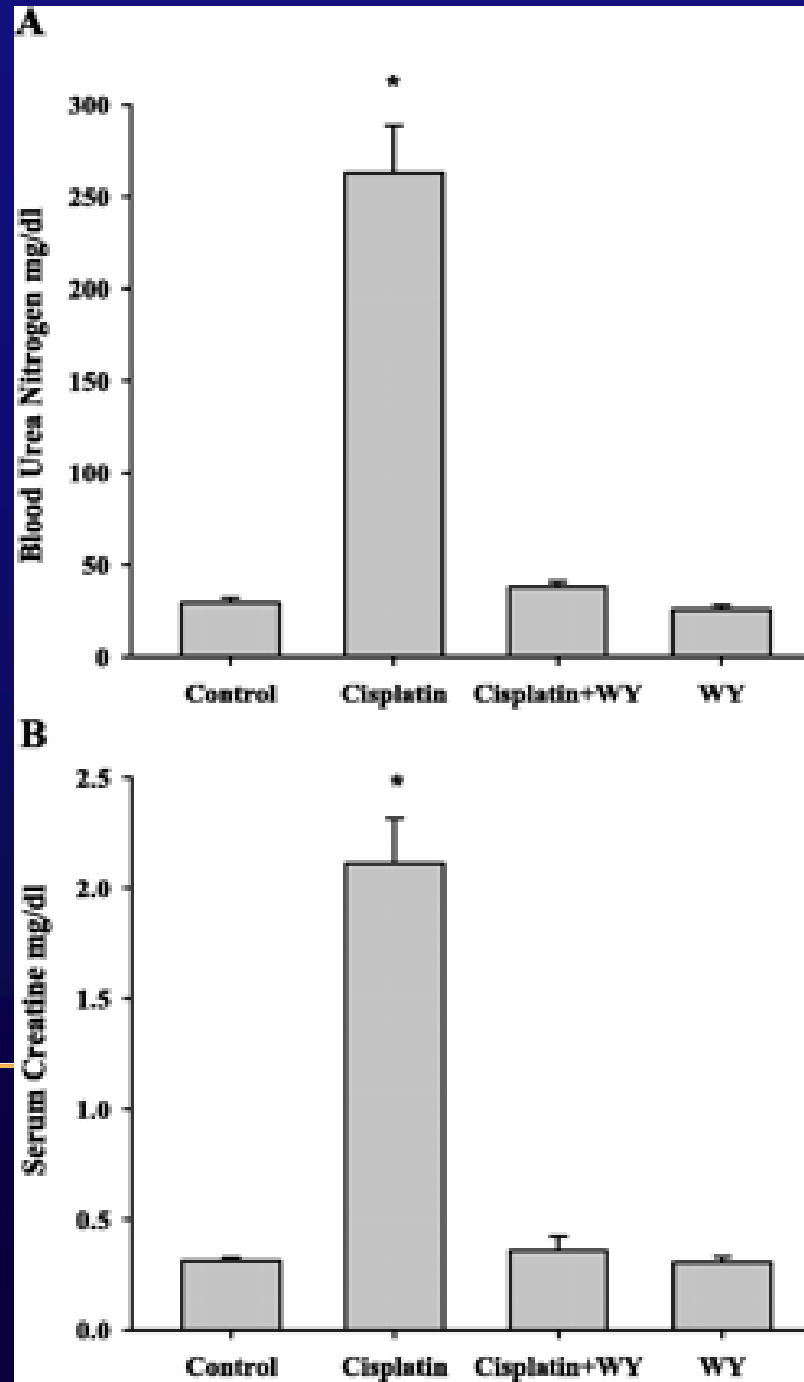
OBJECTIVE

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

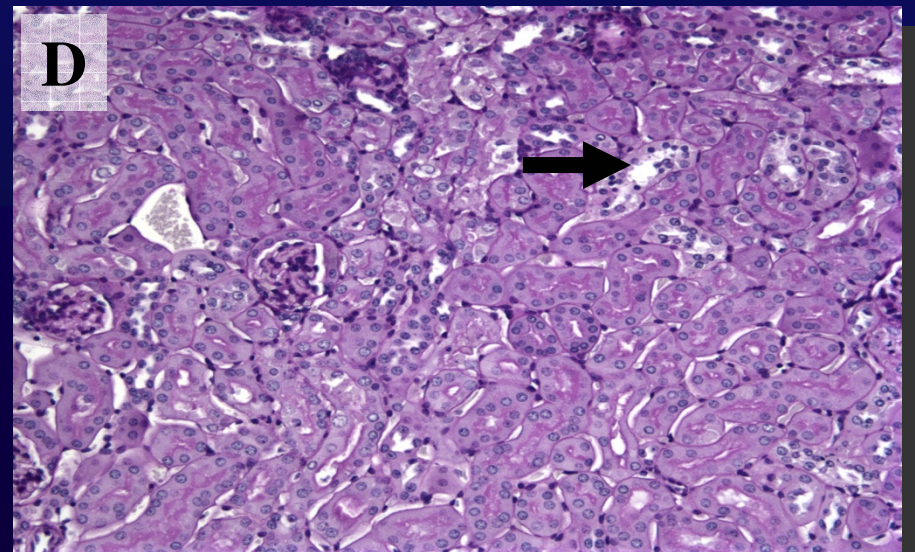
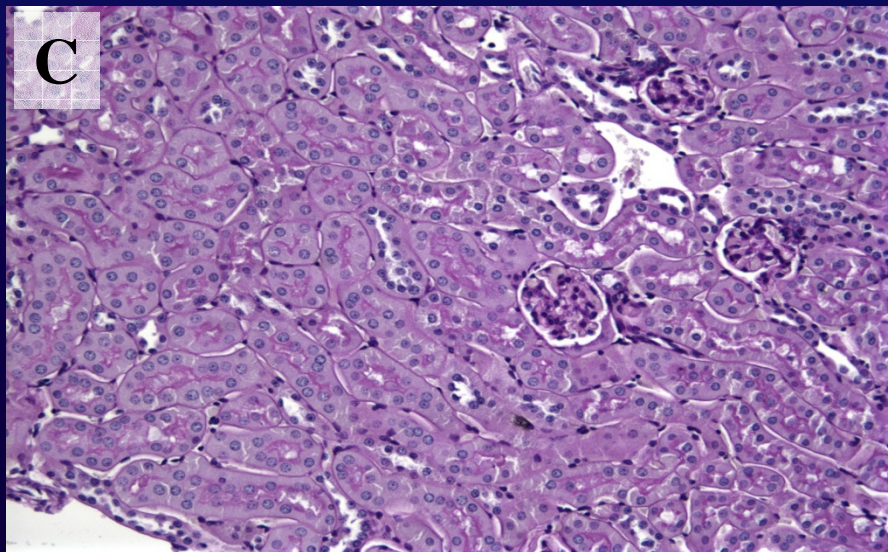
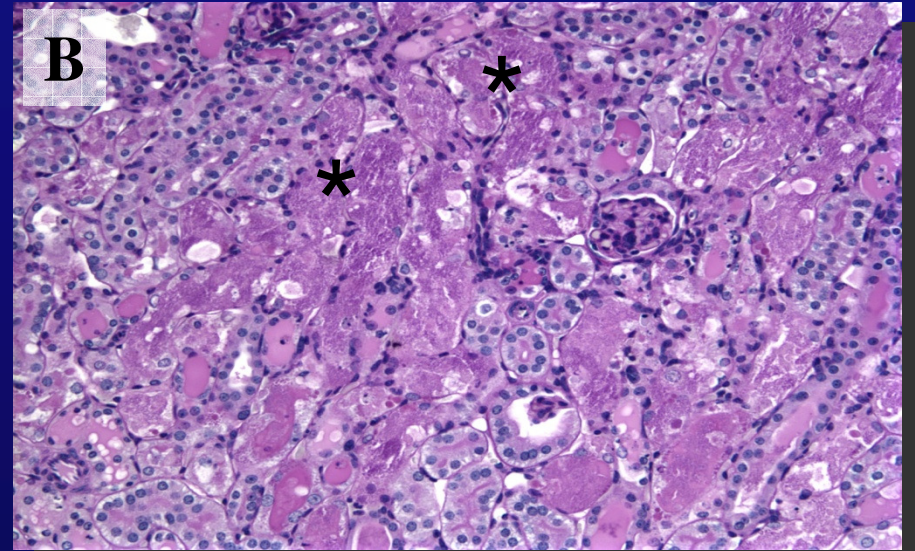
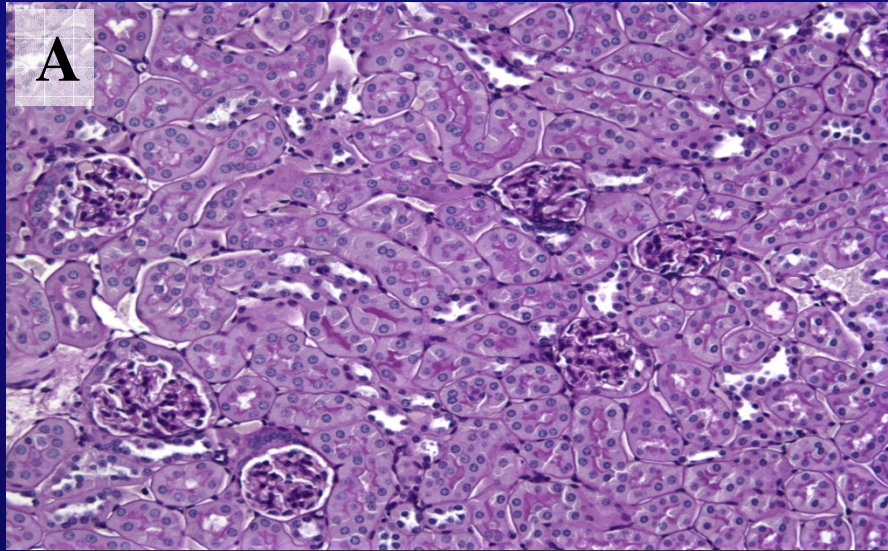
What is the role of endonuclease G (EndoG) in this pathway?

- 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

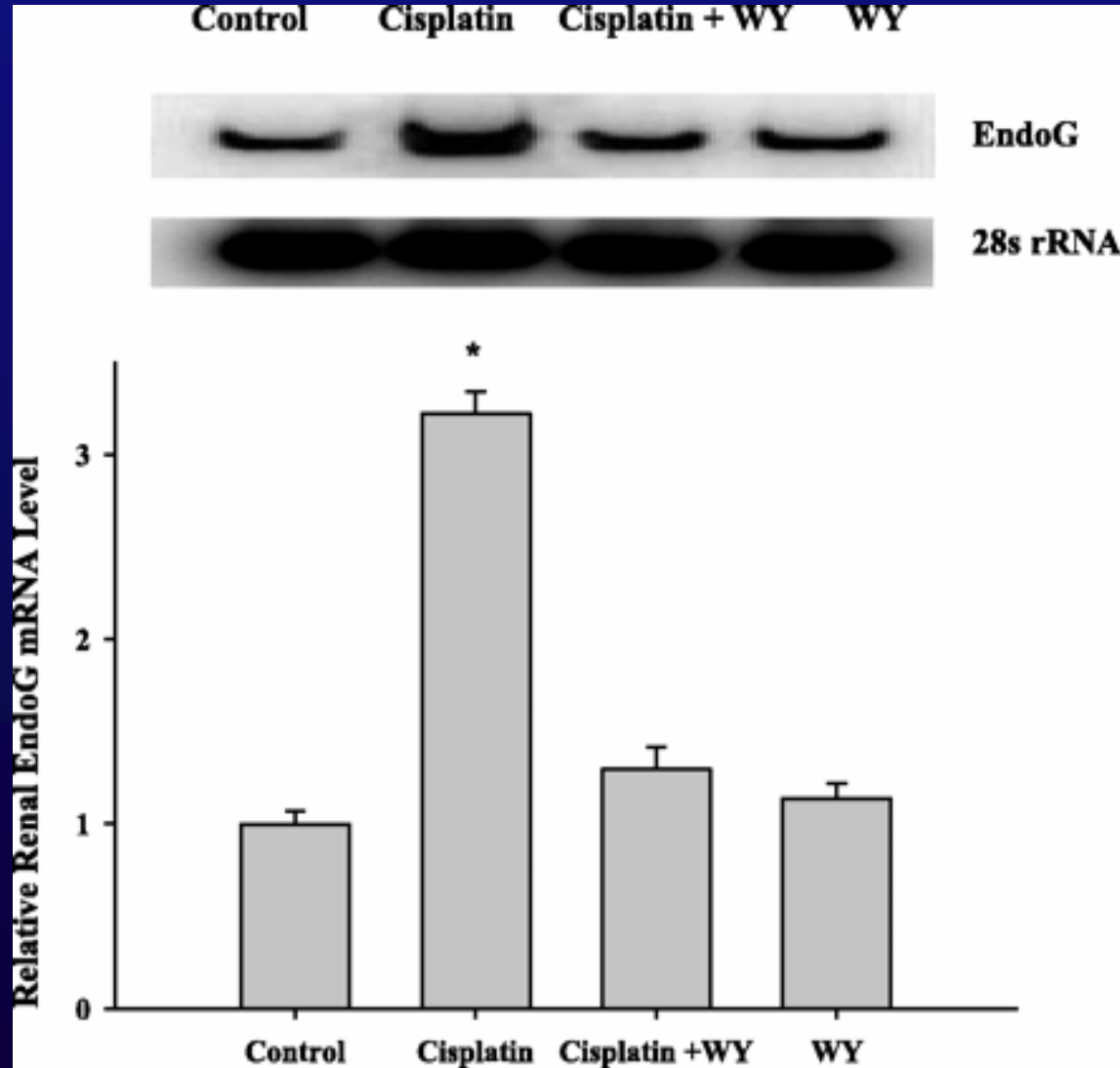


HISTOPATHOLOGICAL ALTERATION IN PPAR α WILD-TYPE MICE



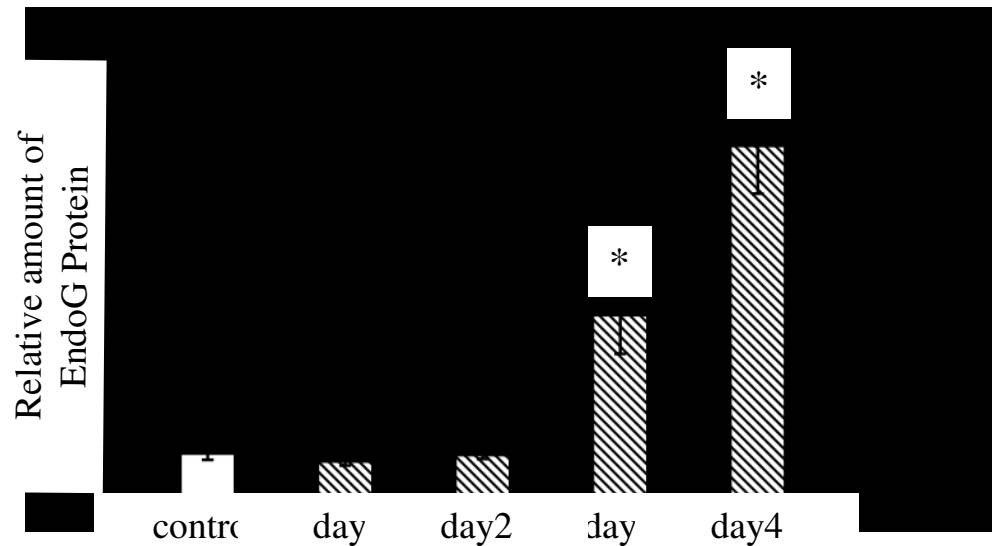
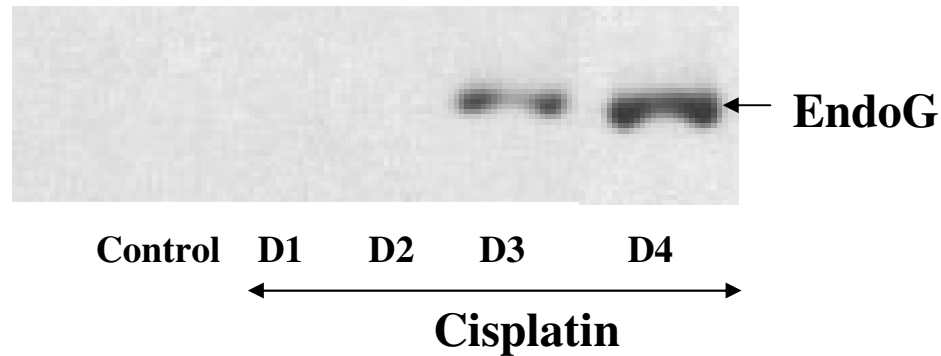
Examine the potential mechanism by which PPAR alpha ligand ameliorates cisplatin-induced ARF:

EFFECT OF WY TREATMENT ON EndoG mRNA LEVELS



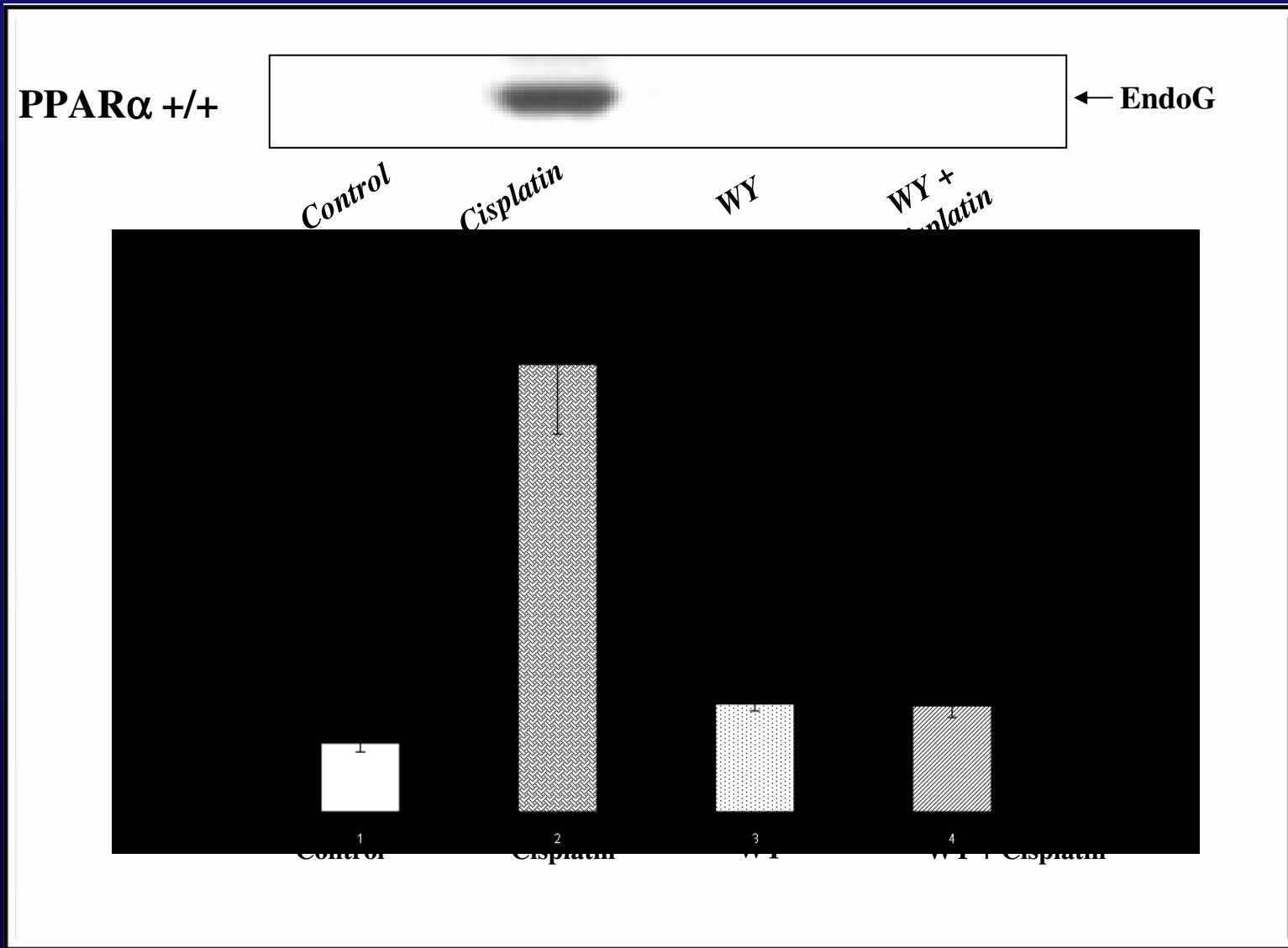
TIME COURSE: EFFECT OF CISPLATIN ON RELATIVE EndoG LEVELS

PPAR α +/+

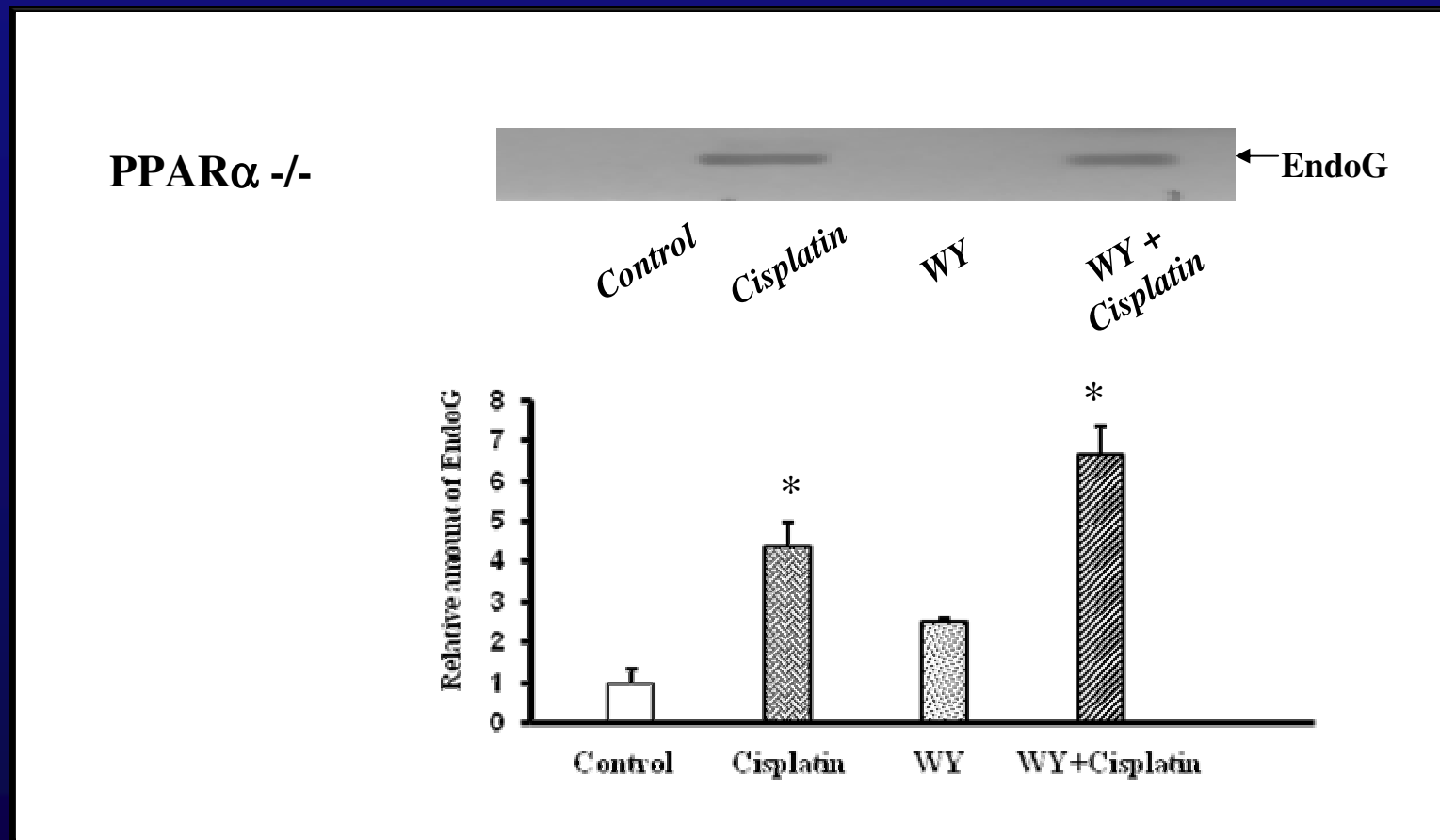


- By day 4 of cisplatin, EndoG protein is increased nearly eight-fold.

EFFECT OF WY TREATMENT ON EndoG LEVELS



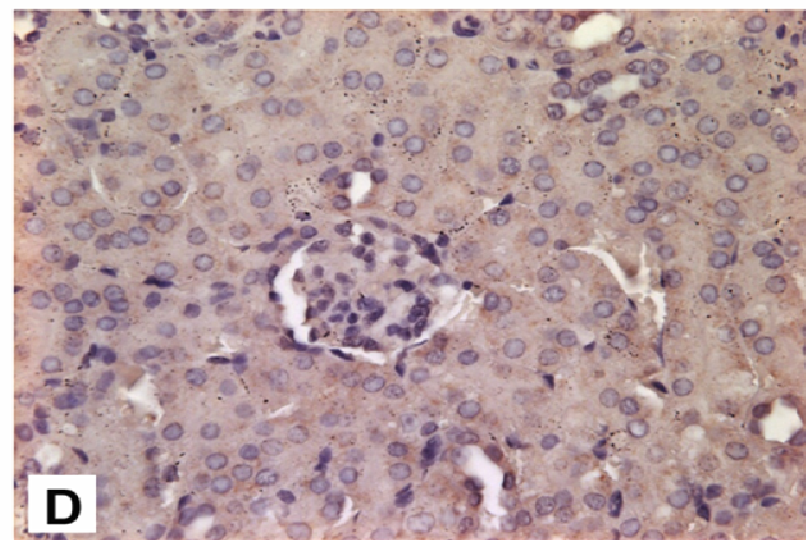
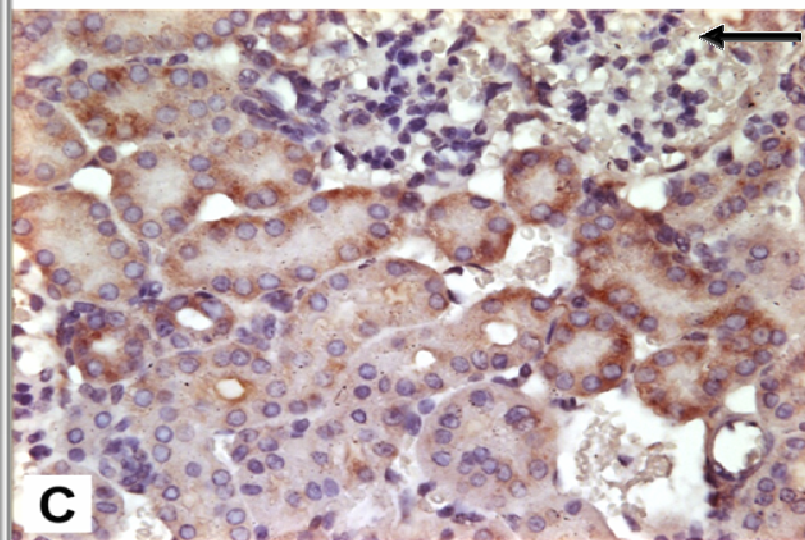
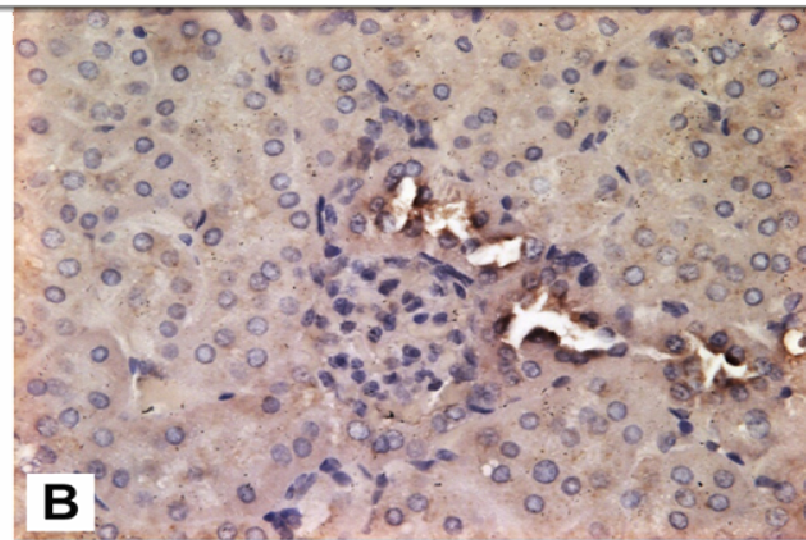
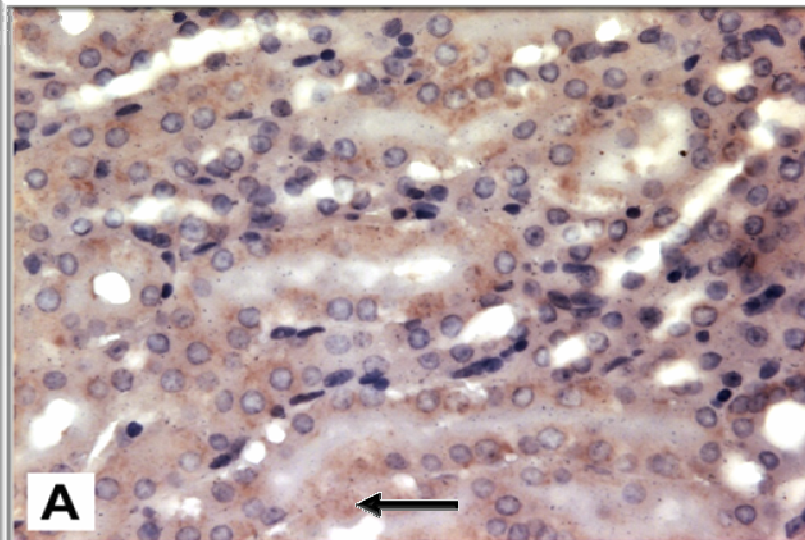
EFFECT OF WY TREATMENT ON EndoG LEVELS



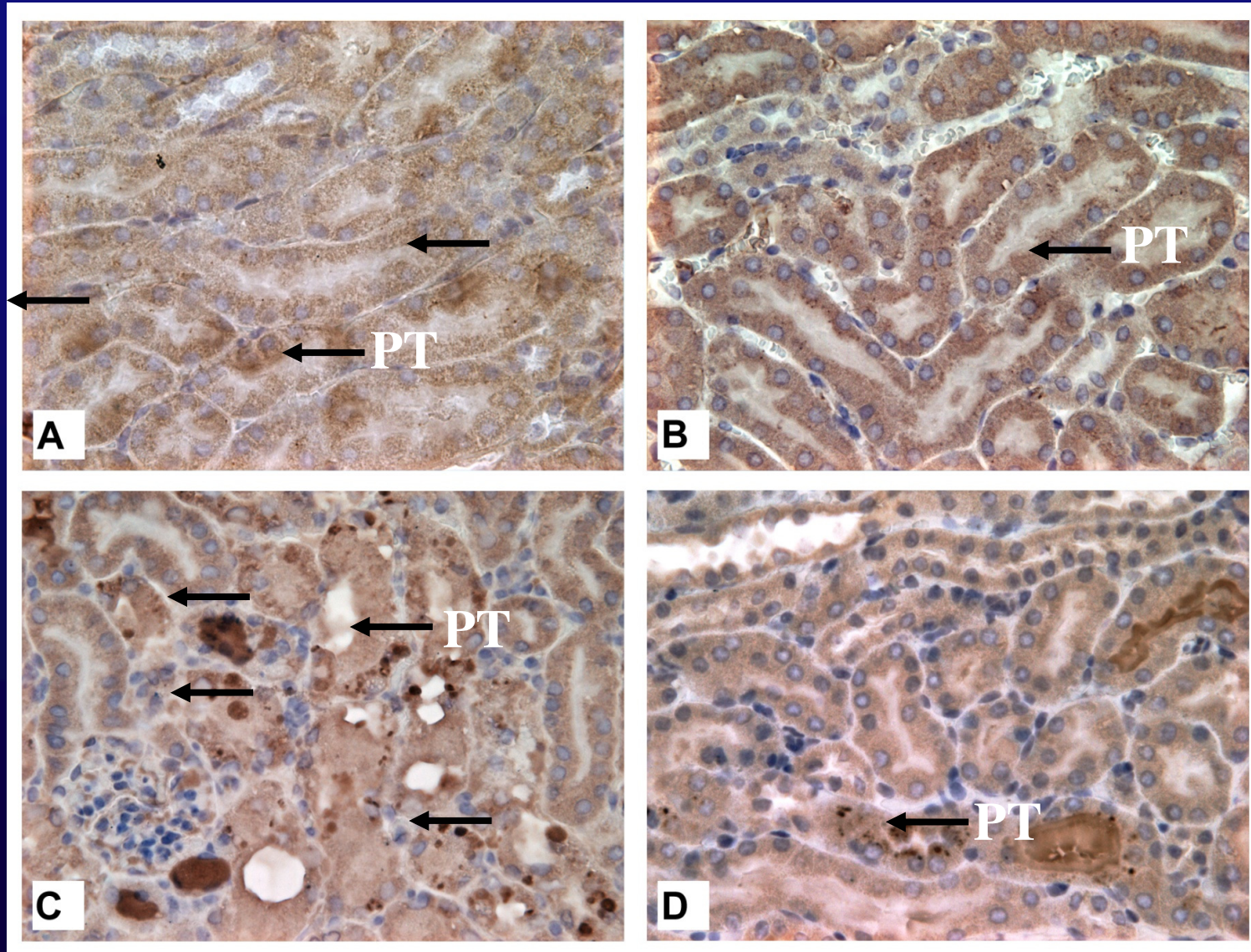
- This rise in EndoG was not blocked by WY in PPAR- α null mice.

Our finding suggests that the protective effect of PPAR- α ligands depends on an intact and functionally active PPAR α gene

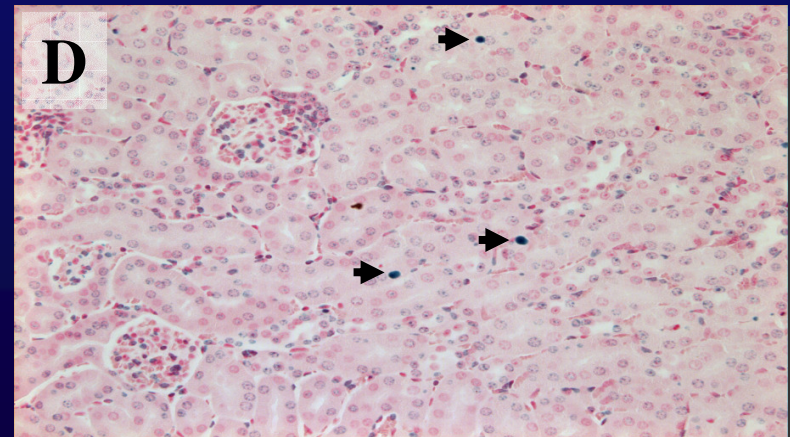
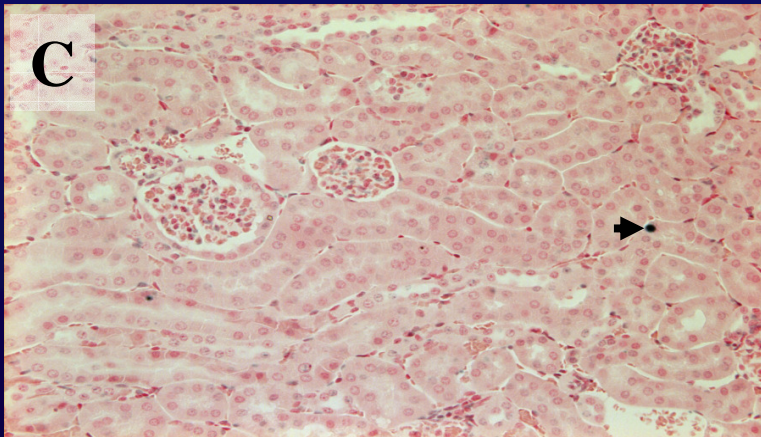
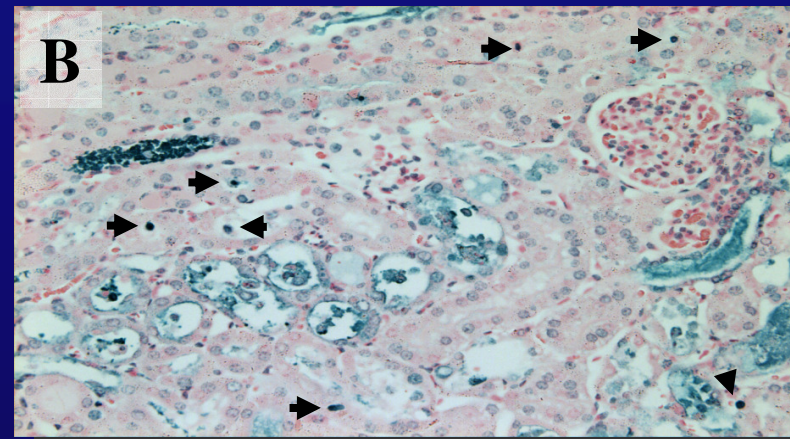
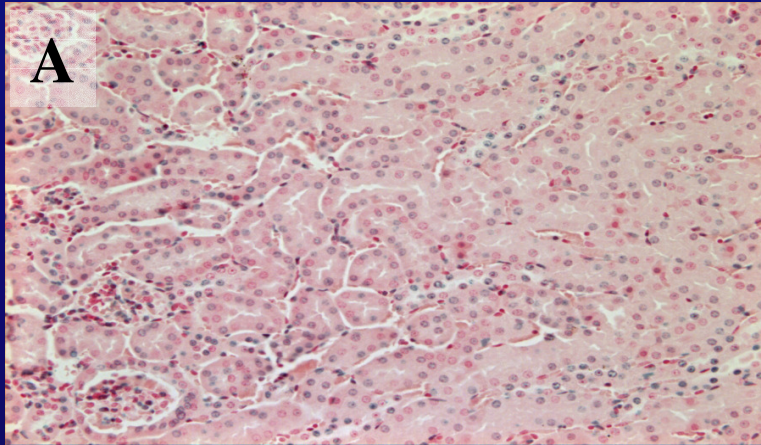
IN SITU HYBRIDIZATION – LOCALIZATION OF EndoG



IMMUNOLocalIZATION OF EndoG

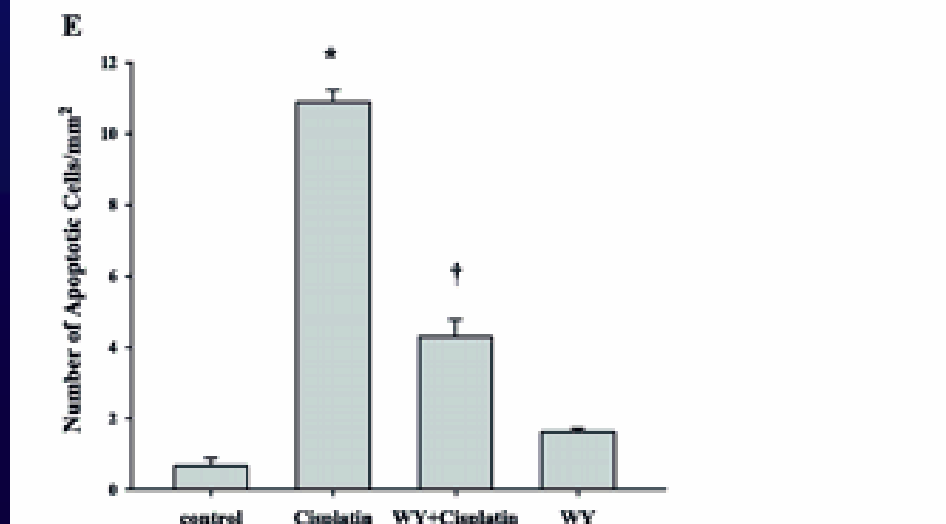
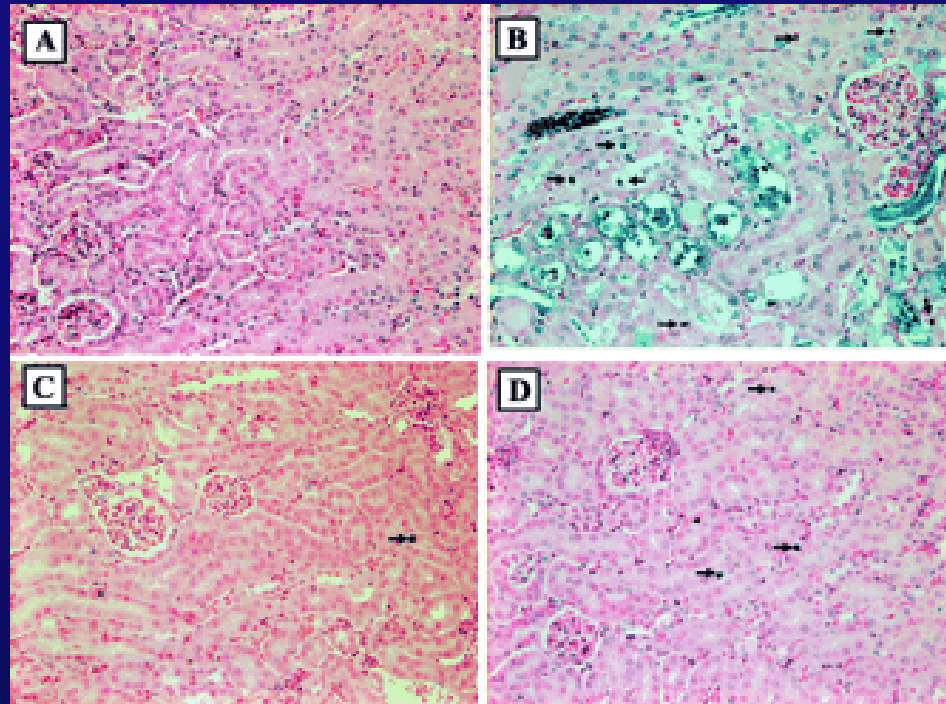


IN SITU EVALUATION OF KIDNEY APOPTOSIS



Terminal deoxynucleotidyl transferase mediated dUTP nick-end-labeling

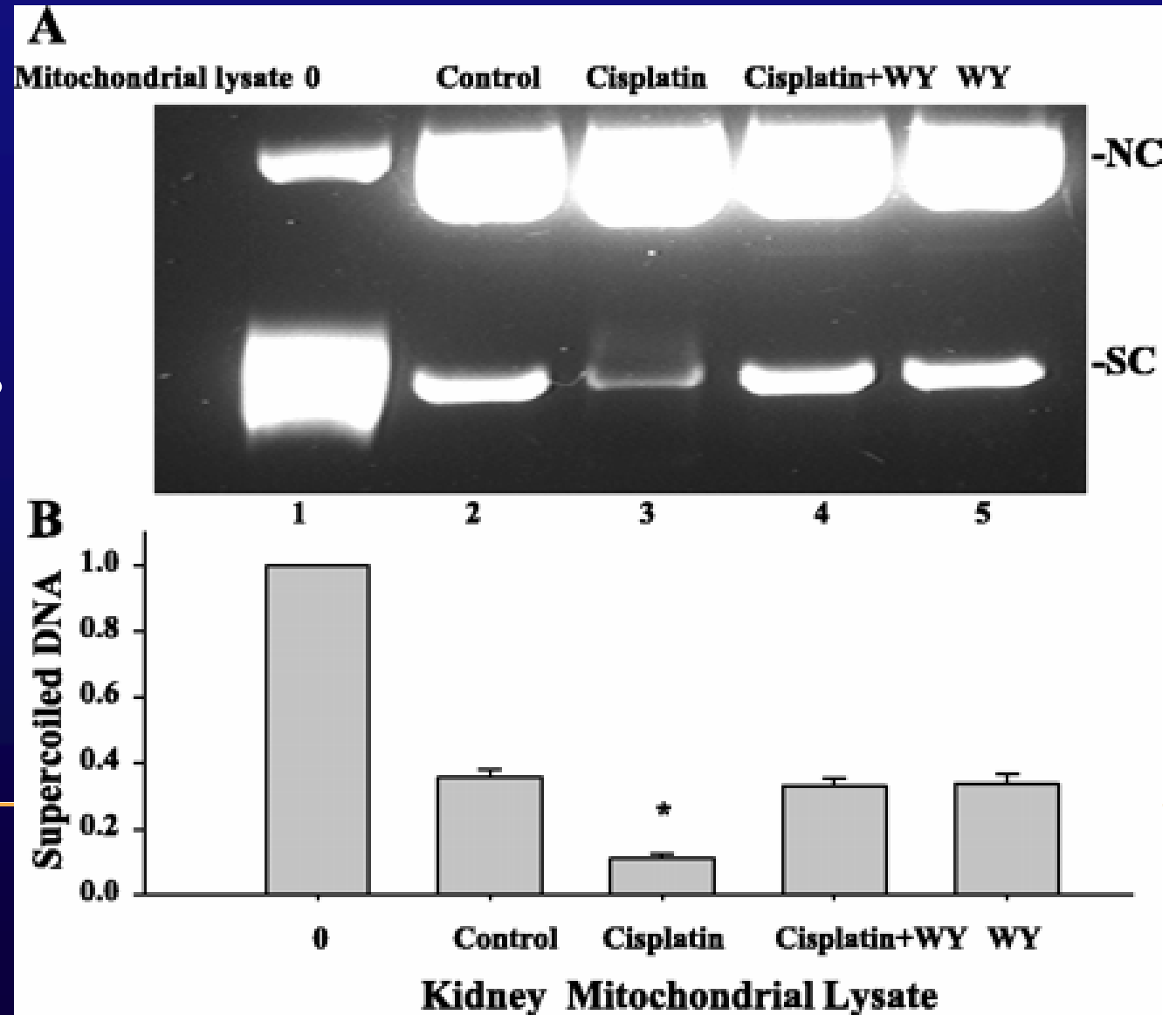
TUNNEL-POSITIVE NUCLEI IN KIDNEY CORTEX

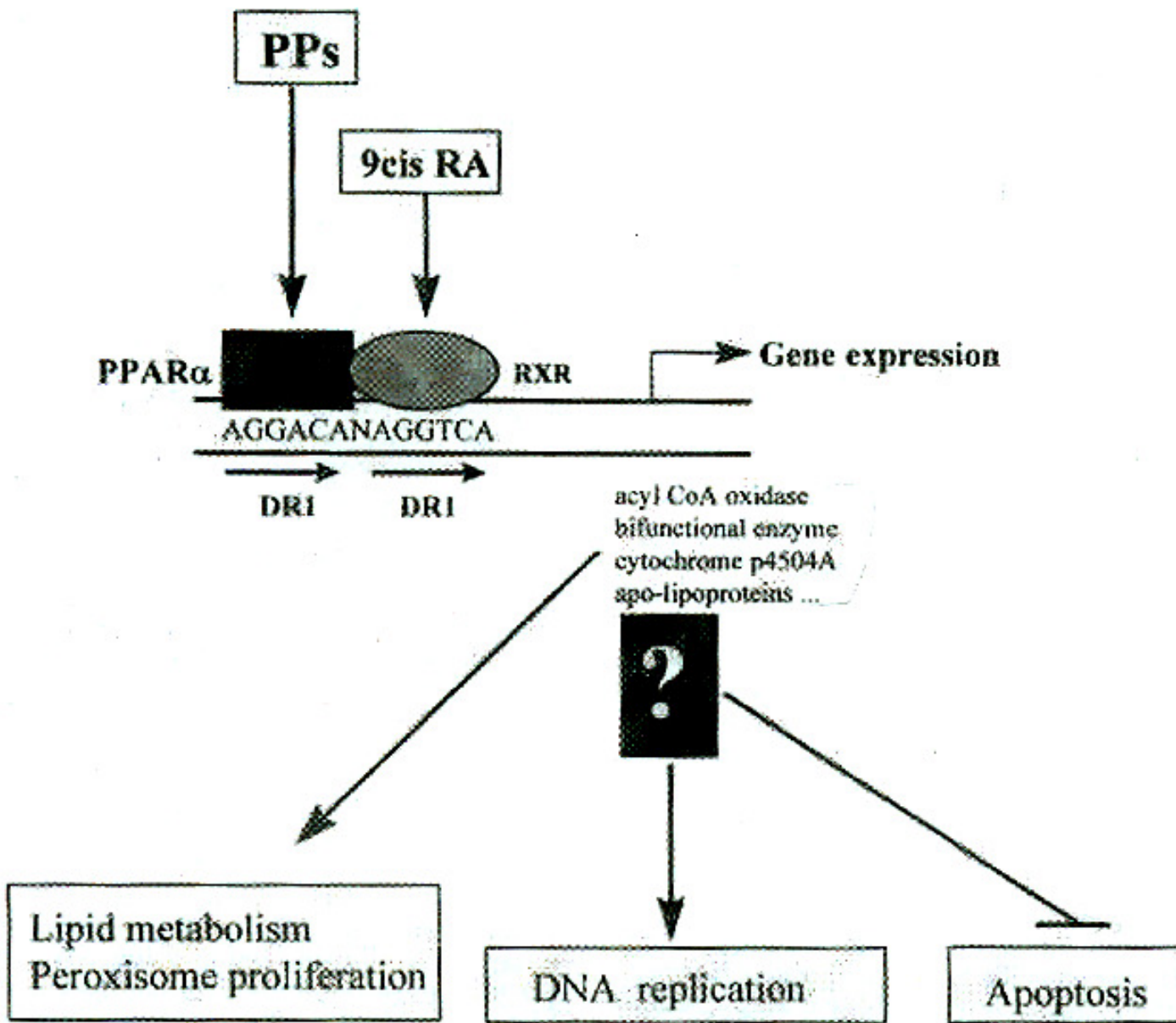


EFFECT OF KIDNEY MITOCHONDRIAL LYSATE ON SUPERCOILED DNA

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

Endonucleolytic nicking
of supercoiled plasmid
DNA





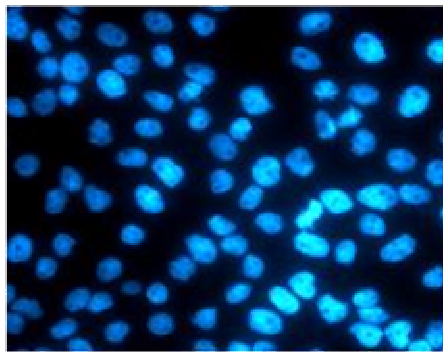
Section II

Fibrates prevents cisplatin-induced proximal tubule cell death : Effect of Bezafibrate

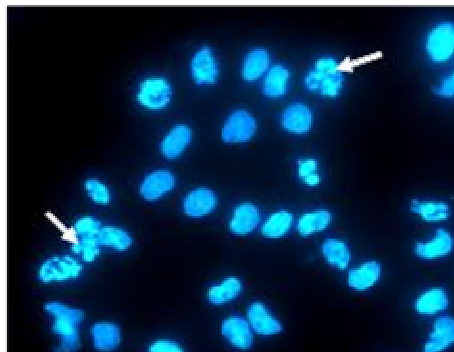
Examined the cellular mechanism by which bezafibrate prevents cisplatin induced proximal tubule cell death

DAPI NUCLEAR STAINING OF RENAL PROXIMAL TUBULE CELLS

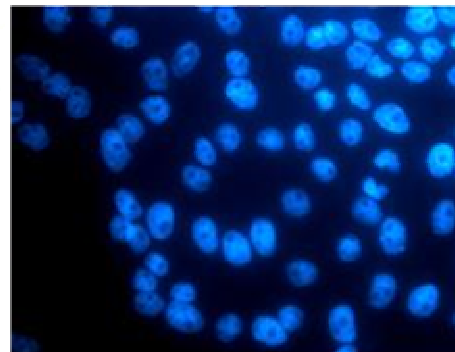
A Control



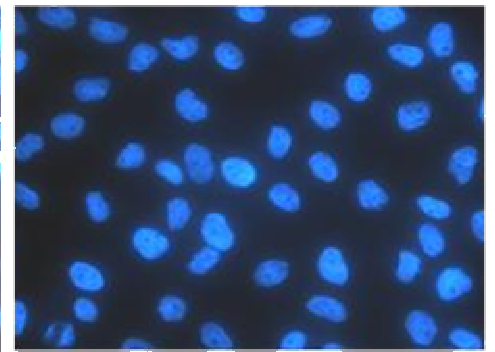
Cisplatin



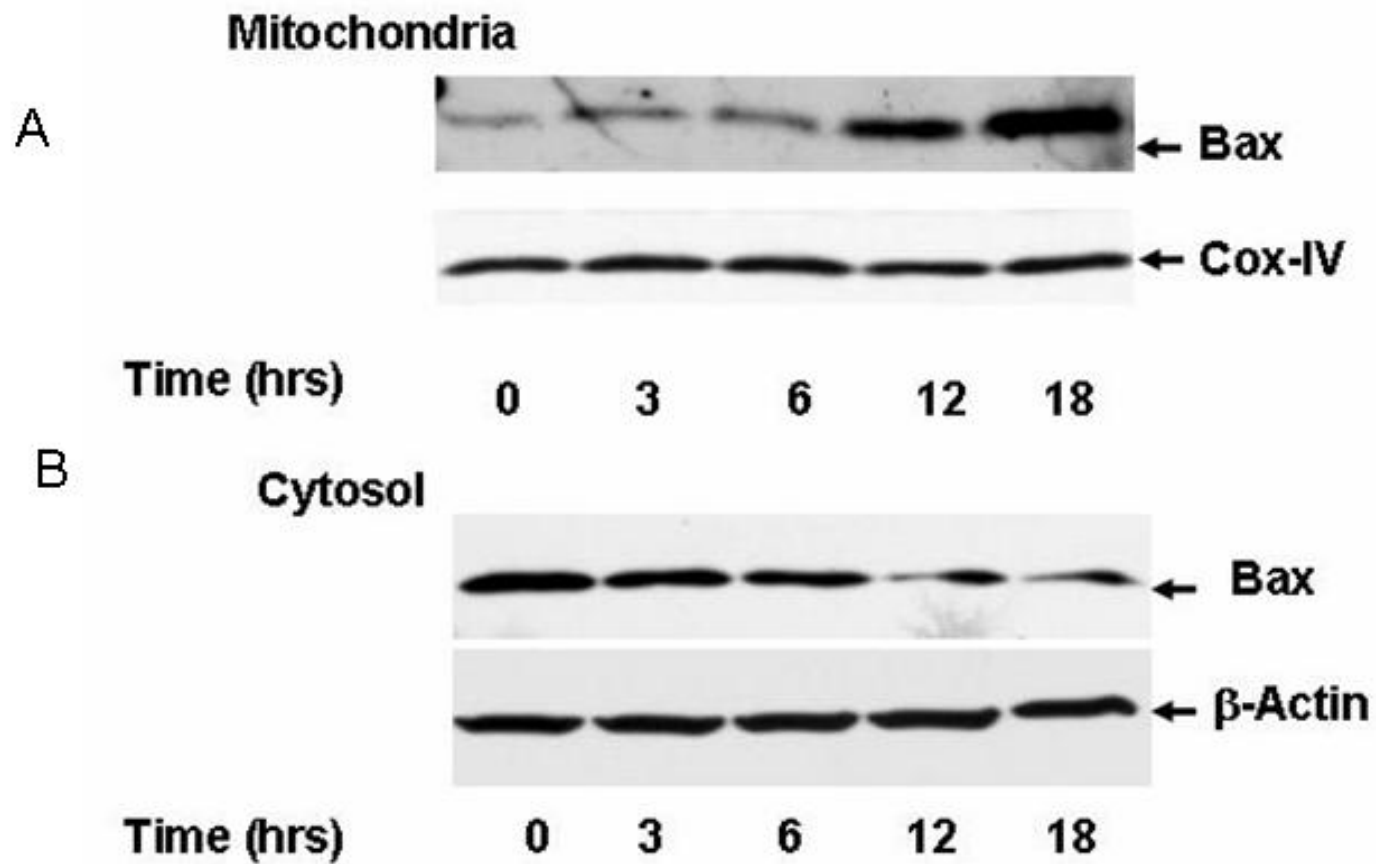
Bezafibrate



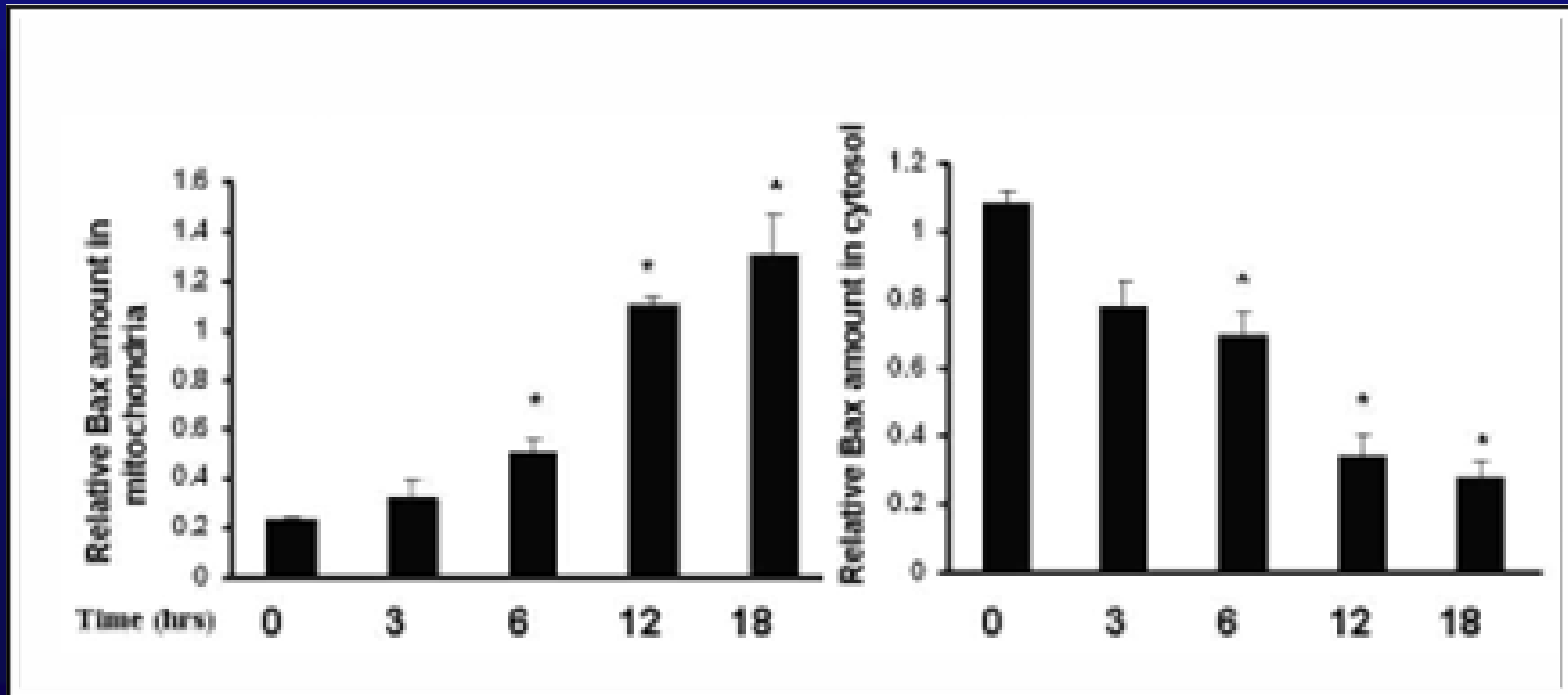
Bezafibrate+
Cisplatin



TIME COURSE EFFECT OF CISPLATIN ON PRO- AND ANTI-APOPTOTIC MOLECULES

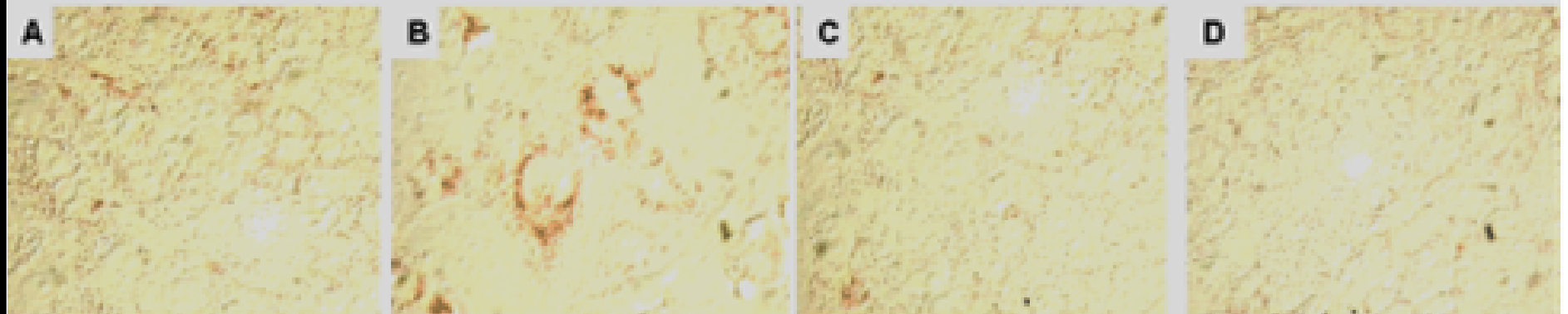


TIME COURSE OF Bax CONCENTRATION AND LOCALIZATION WITHIN THE CELL

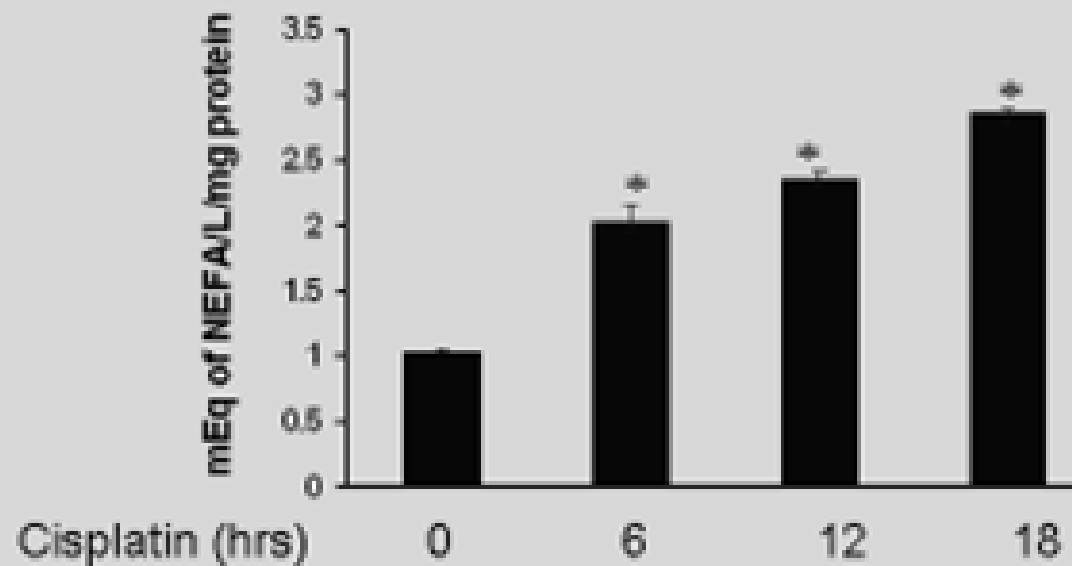


- Cytosolic Bax decreases sharply while mitochondrial Bax increases roughly six-fold.

EFFECT OF BEZAFIBRATE ON ORO STAINING IN RENAL PROXIMAL TUBULE CELLS

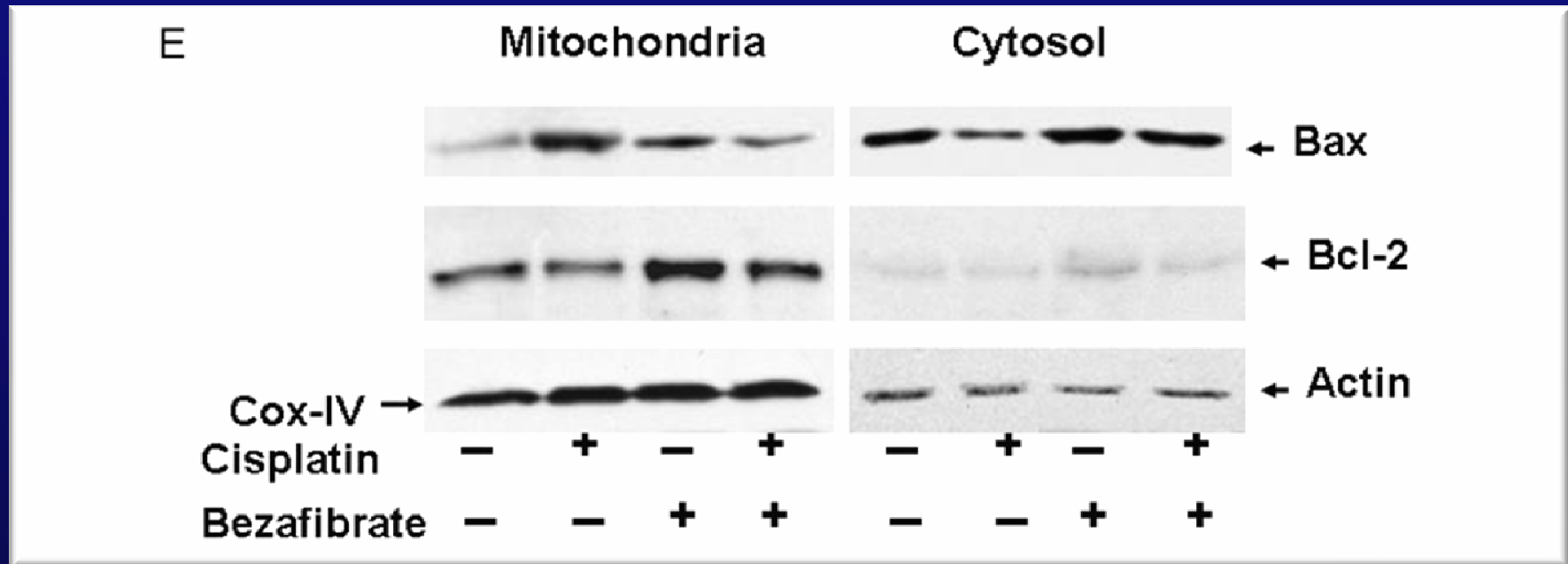


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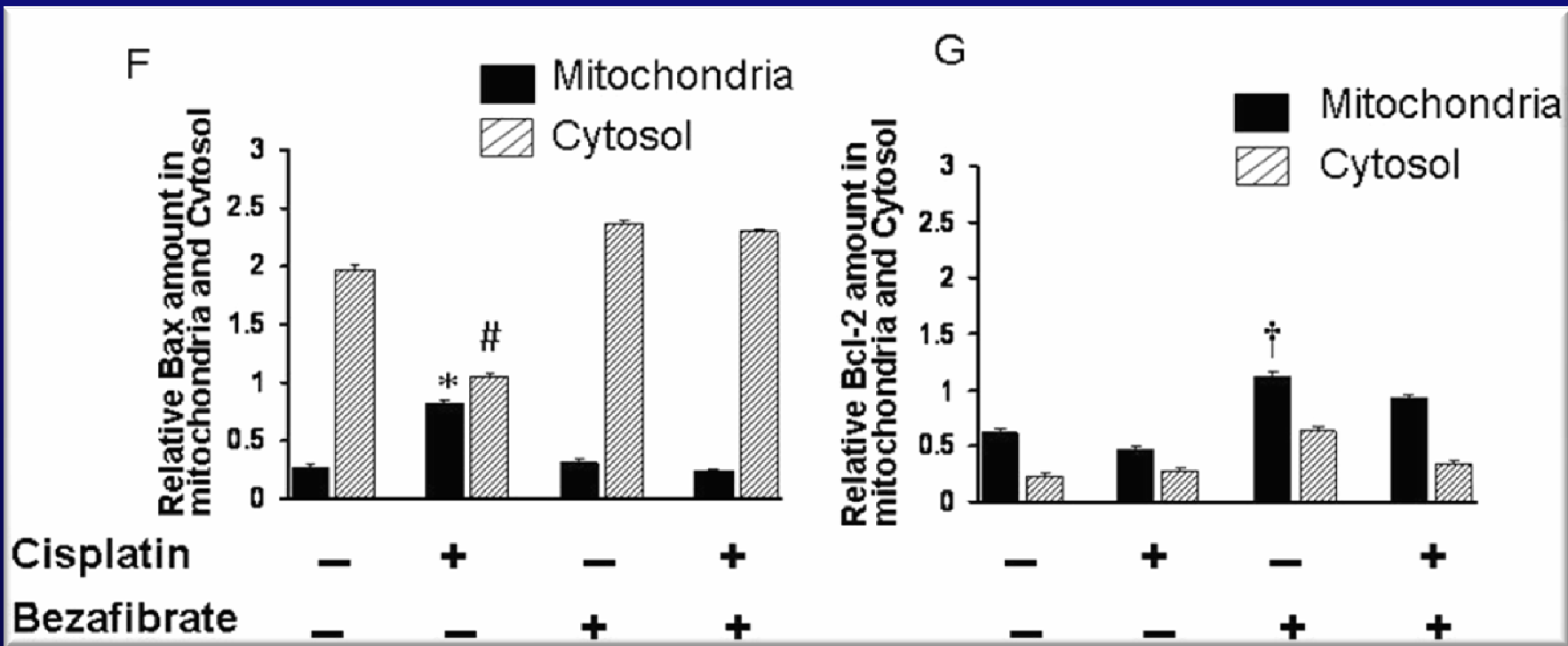
TIME COURSE OF CISPLATIN IN NEFA IN RENAL PROXIMAL TUBULE CELLS

EFFECTS OF BEZAFIBRATE ON Bax AND Bcl-2 LOCALIZATION WITHIN THE CELL AFTER CISPLATIN



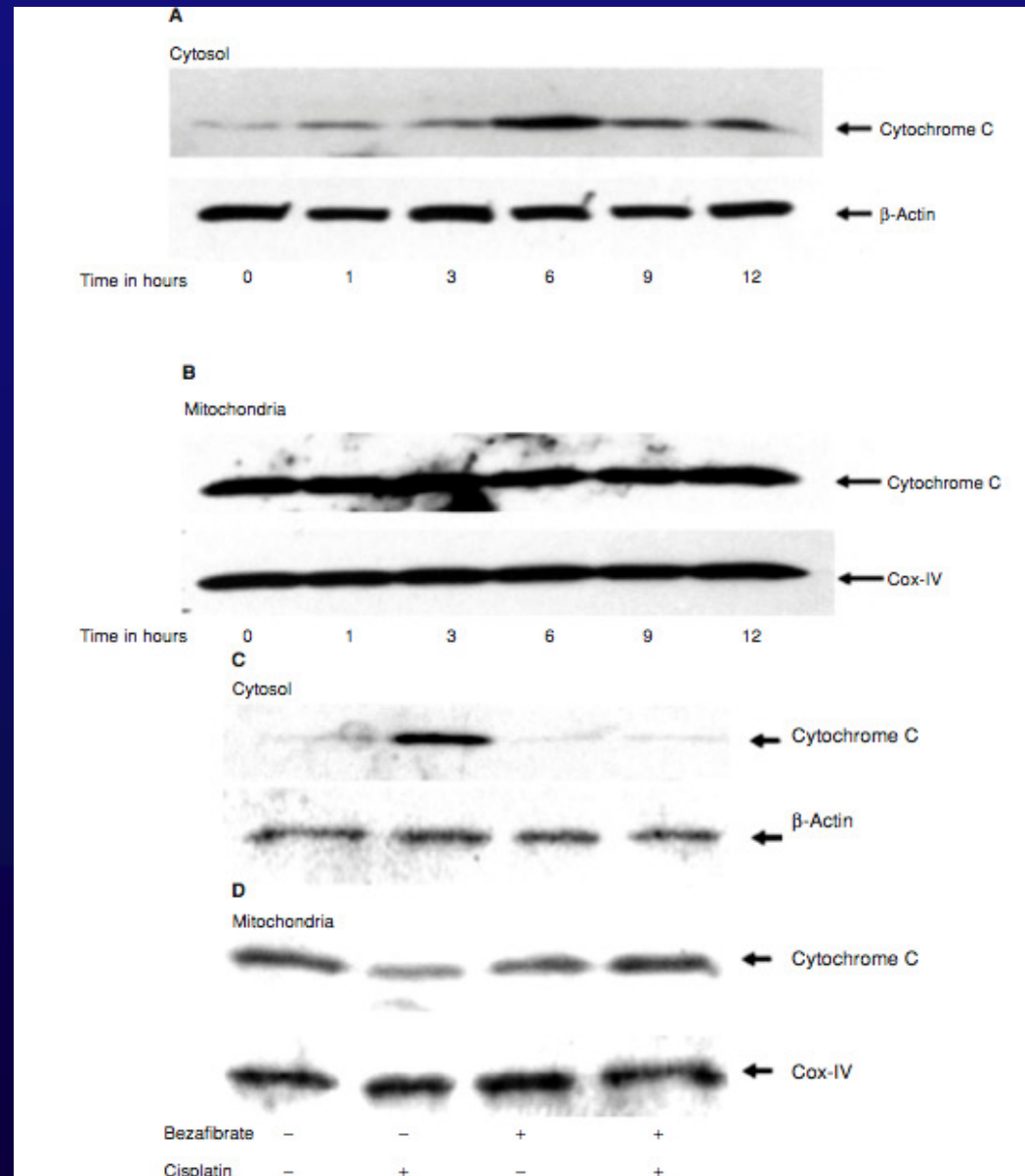
- Bezafibrate treatment prevents Bax migration to the mitochondria.
- This may be due to an increase in cytosolic Bcl-2

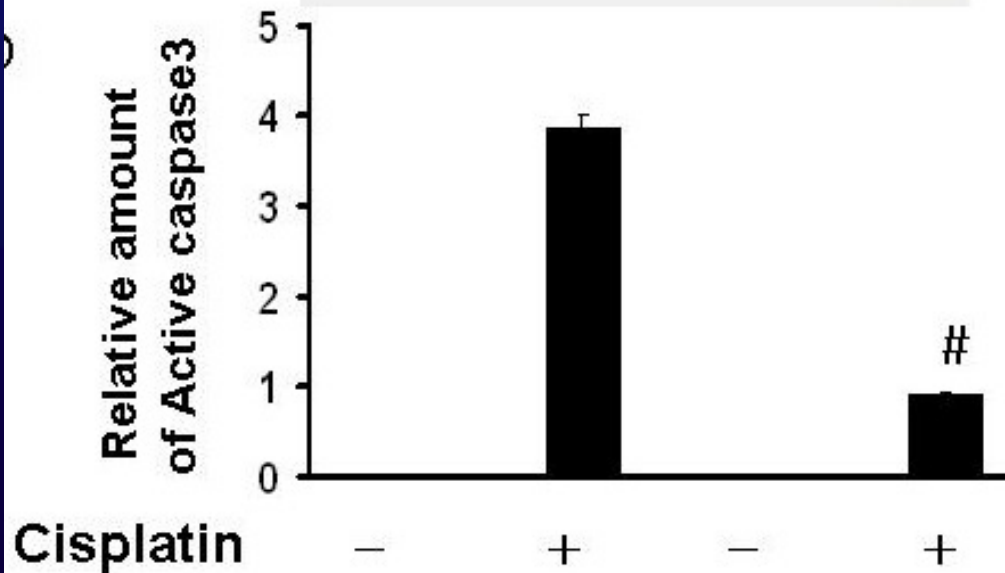
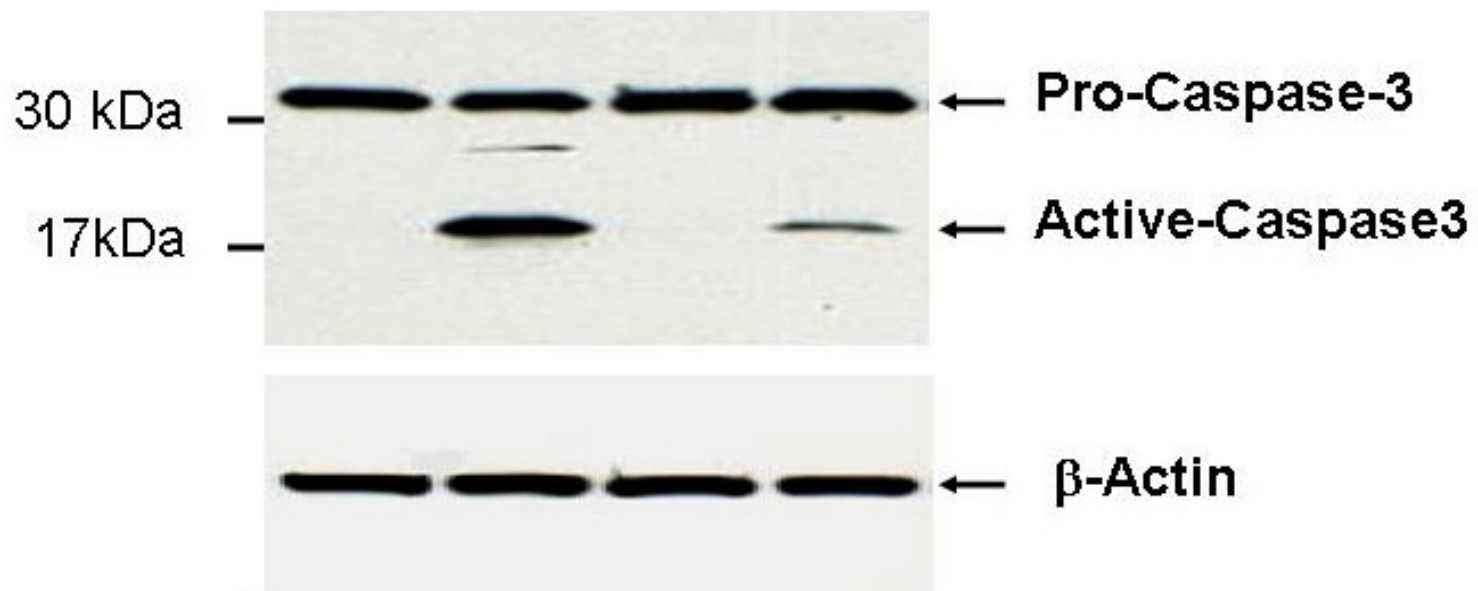
EFFECTS OF BEZAFIBRATE ON Bax AND Bcl-2 LOCALIZATION WITHIN THE CELL AFTER CISPLATIN

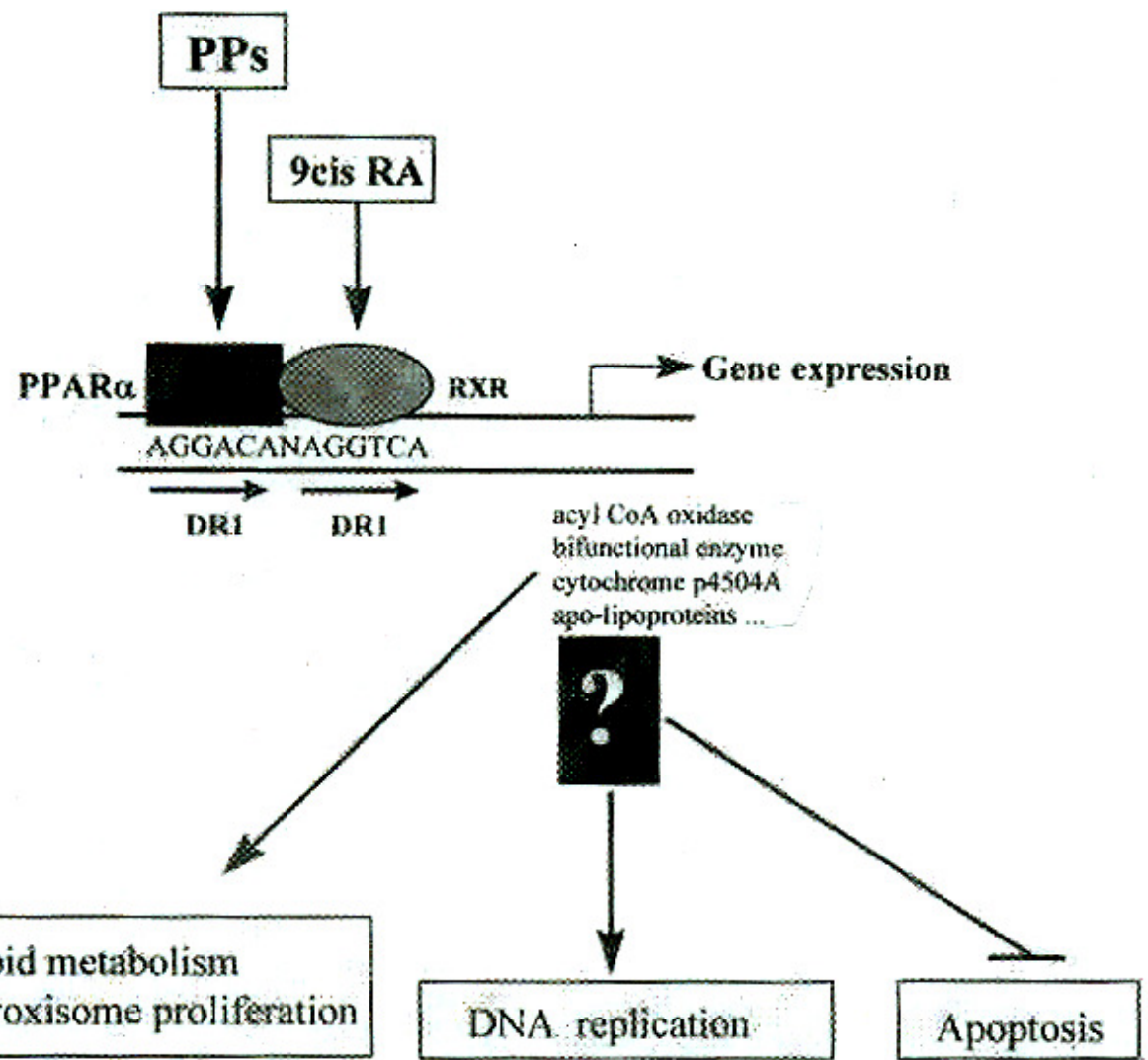


- Bezafibrate treatment prevents Bax migration to the mitochondria.
- This may be due to an increase in cytosolic Bcl-2

Time course of cisplatin induced cytochrome C release



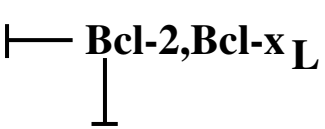




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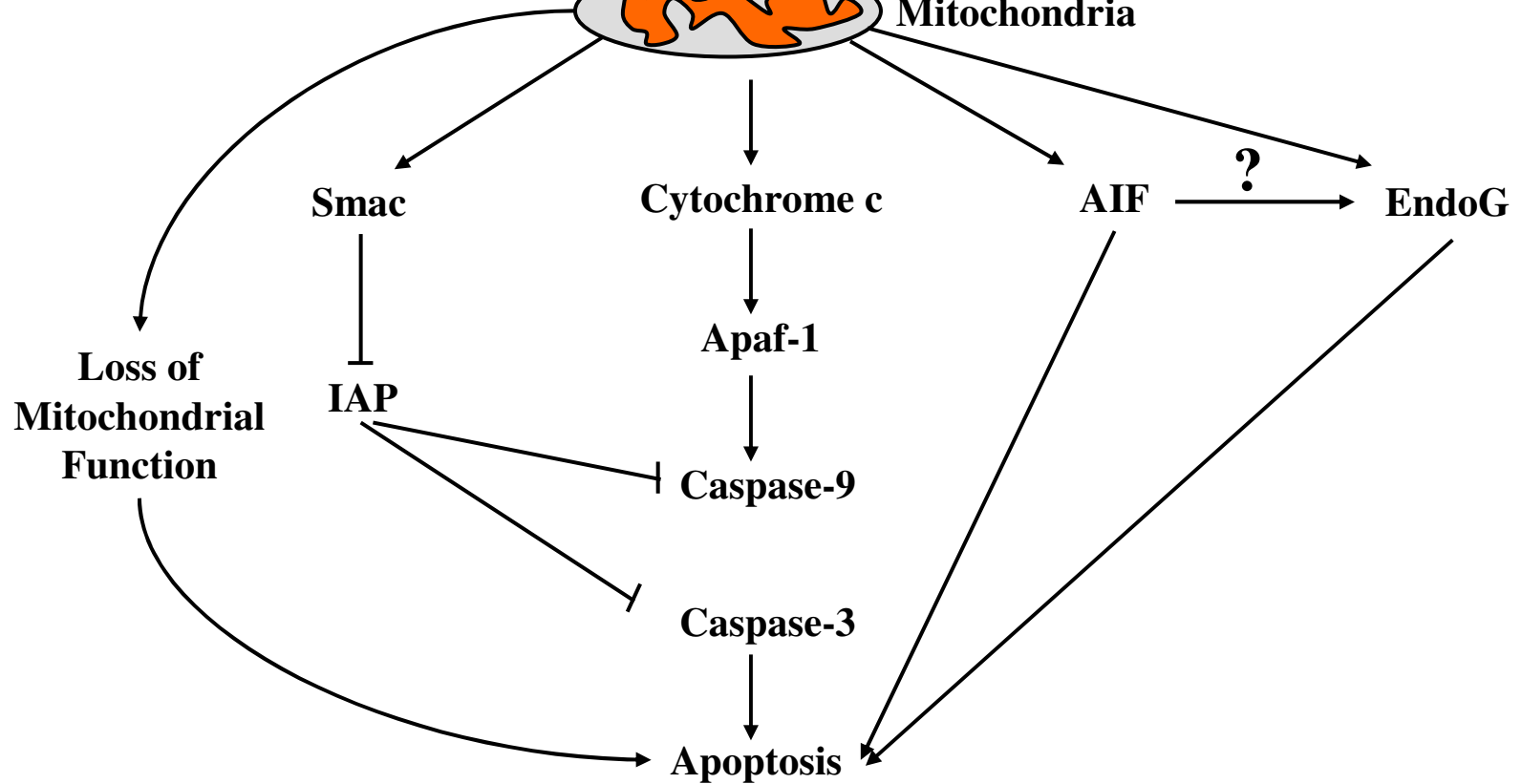


Bcl-2,Bcl-x_L

Bax, Bak,etc.



Mitochondria



CONCLUSIONS

- Our findings 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

ACKNOWLEDGEMENTS

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Nerimen Gokden MD