Heme Oxygenase and oxidative lung injury

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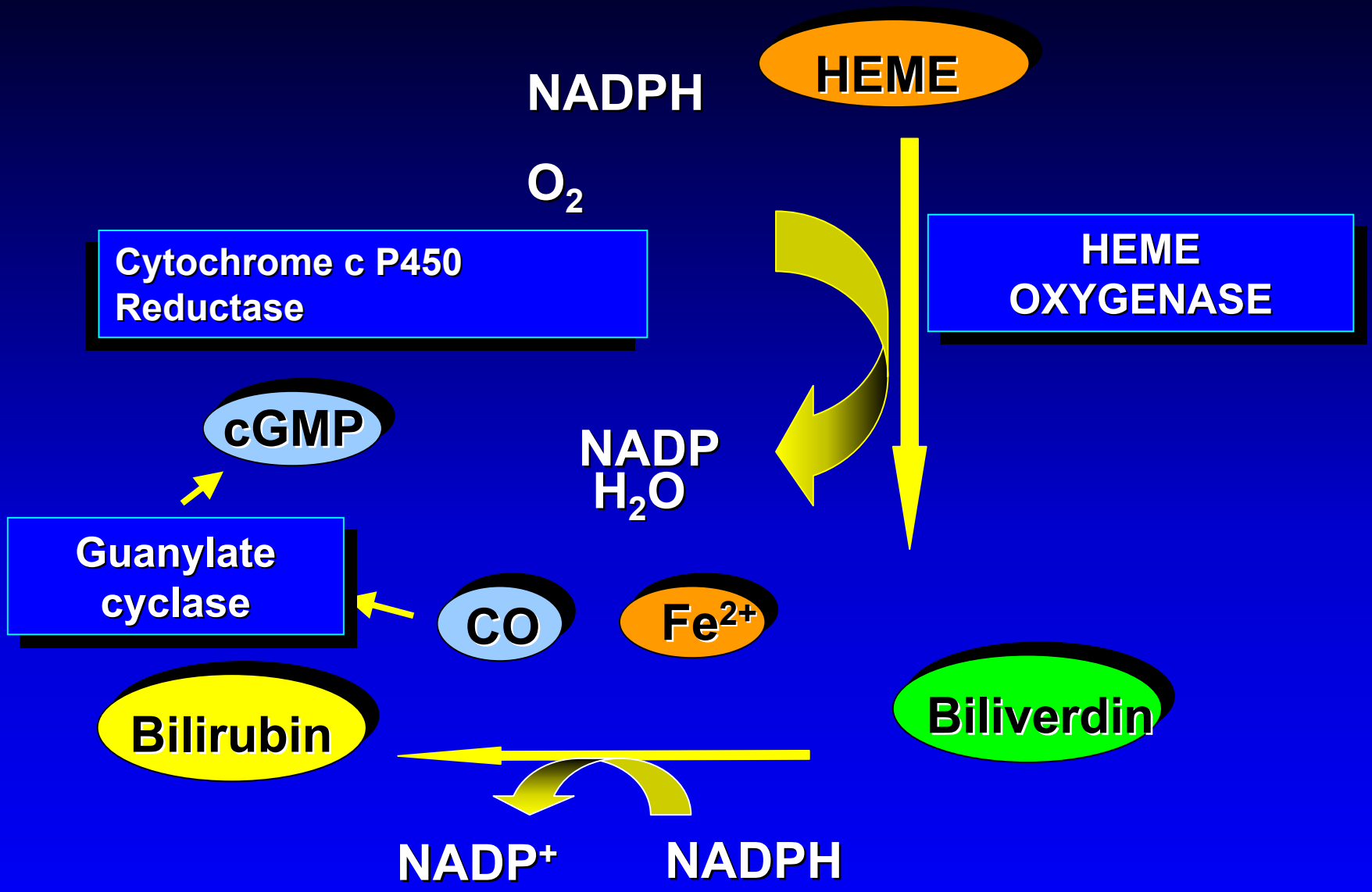
Metabolic Pathway of heme oxygenase

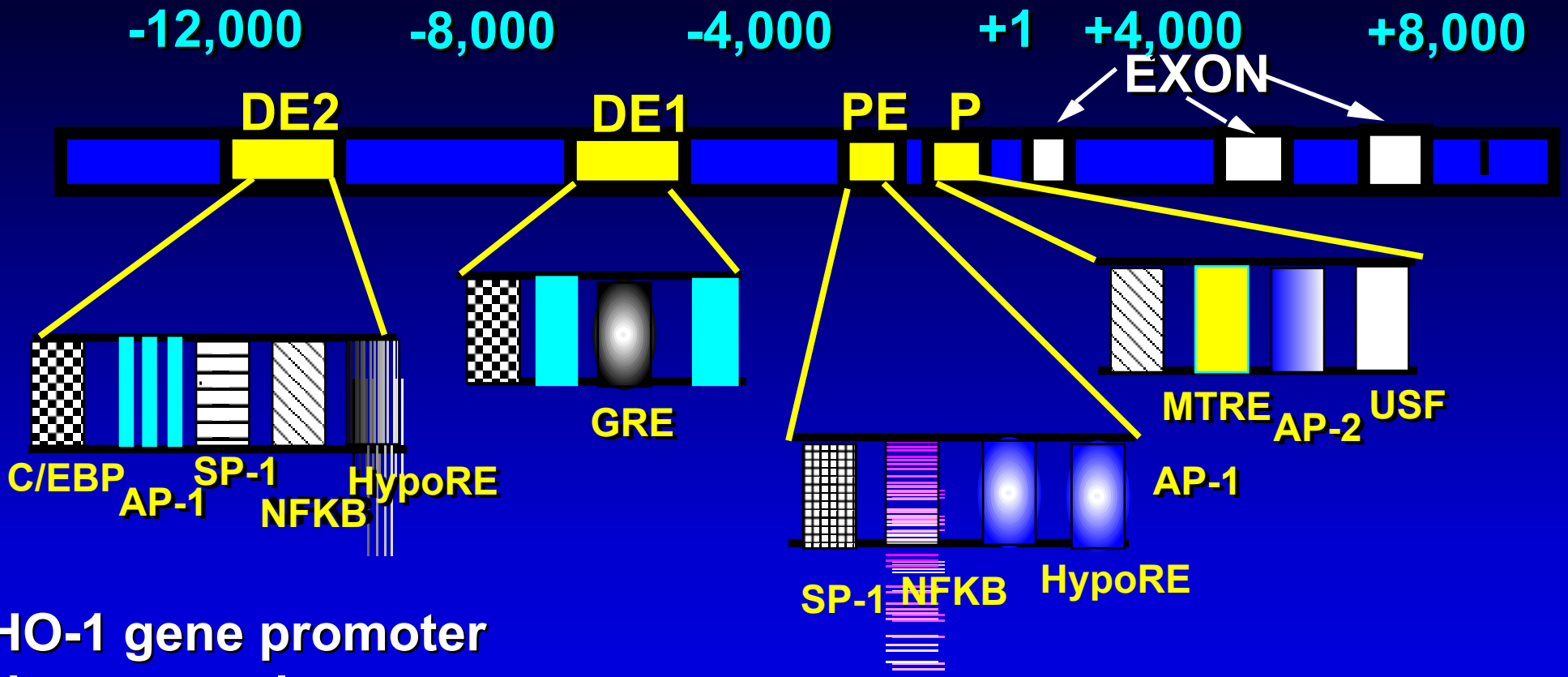


HO Isoenzymes

HO-1	HO-2	HO-3
– Inducible – Multiple regulatory sites – Induction by: • UVA, heavy metals, oxidative stress, inflammation, *etc.* (1-4).	– 'constitutive' – Has a GRE (5) – Induction by • Glucocorticoids (5)	– constitutive – Heme binding (6

1. Applegate LA, *et al*. 1991, *Cancer Res* 51:974-978.
2. Tyrrell RM, *et al*. 1993. *Carcinogenesis* 14:761-765.
3. Shibahara S, *et al*, 1978. *Arch Biochem Biophys* 188:243-250.
4. Janssen YM, *et al*. 1994. *Am J Respir Crit Care Med* 149:795-802.
5. Raju VS, *et al*. 1997. *Biochim Biophys Acta* 1351:89-104.
6. McCoubrey WK, *et al*. 1997. *Eur J Biochem* 247:725-732.

Why is HO-1 so readily inducible ?



- HO-1 gene promoter has several transcription factor binding sites (1-5).

1. Alam J 1994. *J Biol Chem* 269:25049-25056.
2. Alam J, *et al.* 1994. *J Biol Chem* 269:1001-1009.
3. Lee PJ, *et al.* 1996. *Am J Respir Cell Mol Biol* 14:556-568.
4. Lee PJ, *et al.* 1997. *J Biol Chem* 272:5375-5381.
5. Lu TH, *et al.* 2000. *Mol Cell Biochem* 209:17-27.

Heme oxygenase - a general response to oxidative stress.

- There are many examples of the induction of HO-1 in response to an oxidative stress.
- For example, skin fibroblasts demonstrate HO-1 induction after ultraviolet radiation, hydrogen peroxide, menadione or the sulfhydryl reagent sodium arsenite (1).

1. Applegate LA. *et al.* 1991. *Cancer Res* 51:974-978.

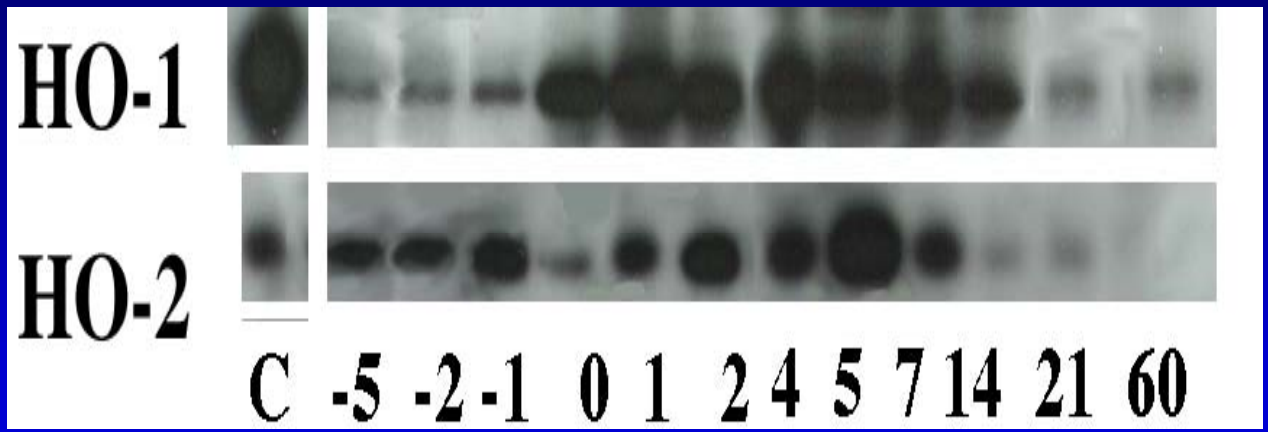
HO in the lung

- In the lung, HO is expressed in many cell types including alveolar macrophages (1), alveolar epithelium (2) and endothelium (3).

1. Harju T, *et al.* 2002. *Respir Med* 96:418-423.
2. Lee PJ, *et al.* 1996. *Am J Respir Cell Mol Biol* 14:556-568.
3. Visner GA, *et al.* 1996. *Am J Physiol* 270:L517-L525.

Lung Developmental Expression of HO

- HO is expressed in the lung throughout development
- Highest levels are in the perinatal period (1)



Representative Western blot of HO-1 and HO-2 immunoreactive protein in the lungs of rats at various ages. -5 to -1 indicate the days before birth. 0 indicates the day of birth.

1. Dennery PA, Rodgers PA. 1996. *J Perinatol* 16:S79-83.

Specific examples:
HO in airway inflammation

- Lung macrophages induce HO-1 after hemin induction (1)
- HO-1 is induced in the alveolar macrophages of asthmatics (2,3)

1. Shibahara S, *et al.* 1978. *Arch Biochem Biophys* 188:243-250.
2. Lim S, *et al.* 2000. *Am J Respir Crit Care Med* 162:1912-1918.
3. Harju T, *et al.* 2002. *Respir Med* 96:418-423.

Specific examples:
HO and environmental toxicants

- **Ozone:** Induction (1,2) or not (3) was observed.
  This may indicate a model specific effect.
- **Asbestos:** Different particles have different effects (4)
  - crocidolite: no effect
  - chrysotile: induction

1. Takahashi Y, *et al.* 1997. *Biochem Pharmacol* 53:1061-1064.
2. Hisada T, *et al.* 2000. *Eur J Pharmacol* 399:229-234.
3. Cosma G.1992. *Toxicol Appl Pharmacol* 117:75-80.
4. Janssen YM. 1994. *Am J Respir Crit Care Med* 149:795-802.

Specific examples: HO in hyperoxia

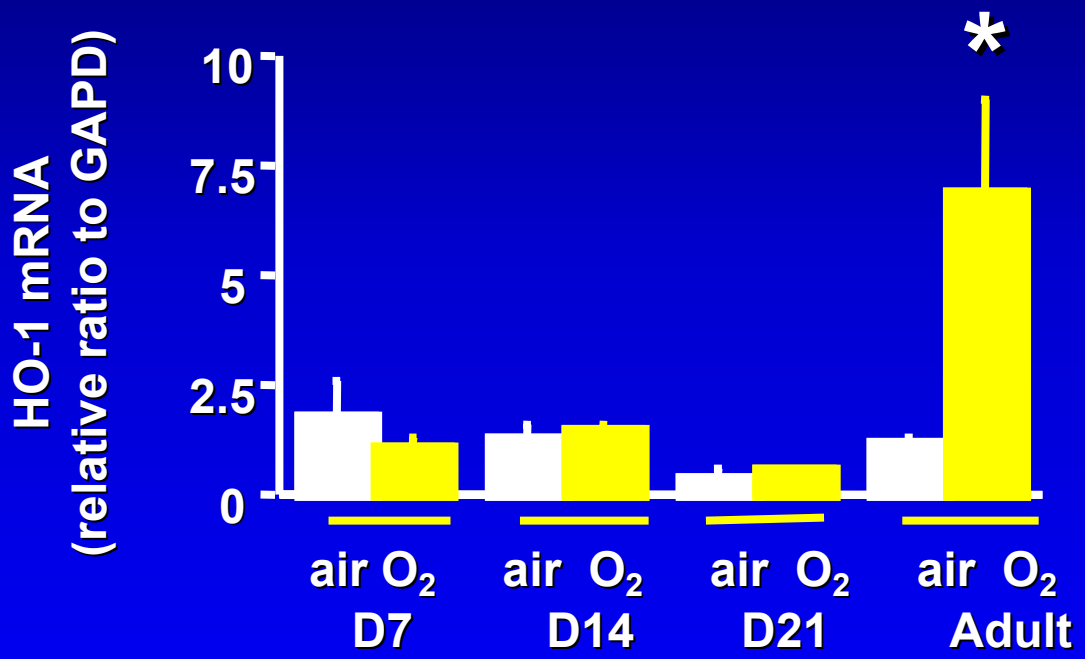
- Increased HO-1 transcription after hyperoxic exposure in adult rodent models (1,2)

1. Lee PJ, *et al*. 1996. *Am J Respir Cell Mol Biol* 14:556-568.
2. Choi AM, *et al*. 1995. *Am J Respir Cell Mol Biol* 13:74-82.

Specific examples: HO-1 in hyperoxia (cont'd)

- However, no increase in HO-1 transcription in the neonatal rodent (1).

1. Dennery PA, *et al.* 1996. *Pediatr Res* 40:815-821.



HO-1 mRNA levels after a 72 hours hyperoxic exposure in rats at various gestational ages [7 days-adult (60 days)] values are the mean ± SE of 6 measurements. * $p < 0.05$ vs. air exposed controls.

Hyperoxic regulation of HO-1 is different in the neonate as compared to adults.

- In neonates exposed to hyperoxia, compared to adults:
  - Increased overall expression of HO-1 protein, increased activity (1,2).
  - Decreased transcriptional regulation of HO-1 mRNA (1,2).
  - This is possibly related to decreased transcriptional factor activation (AP-1) (3).

1. Dennery PA, *et al.* 1996. *Pediatr Res* 40:815-821.
2. Dennery PA, 2000. *Curr Top Cell Regul* 36:181-199.
3. Yang G, *et al.* 2000. *Am J Physiol Lung Cell Mol Physiol* 278:L393-398.

Role of HO in the lung: HO as an antioxidant

- HO-1 cDNA transfection protects against:
  - heme mediated injury (1,2)
  - oxygen toxicity (3-4) and H_2O_2 (1)
- HO-1 antisense transfection aggravates
  - oxygen toxicity (3)
  - UVA radiation (5)
- HO-2 is also protective in the lung (6)

1. Abraham NG, *et al.* 1995. *Invest Ophthalmol Vis Sci* 36:2202-2210.
2. Yang L, *et al.* 1999. *Am J Physiol* 277:L127-133.
3. Dennery P, *et al.* 1997. *J. Biol Chem* 272:14937-14942.
4. Otterbein LE, *et al.* 1999. *J Clin Invest* 103:1047-1054.
5. Vile GF, 1994. *Proc Natl Acad Sci USA* 91:2607-2610.
6. Dennery PA, *et al.* 1998. *J Clin Invest* 101:1001-1011.

What is protective about HO?

- CO (one CO molecule is released from each heme, slide 2)
  - Neurotransmitter (1)
  - Vasodilator (2)
  - Bronchodilator (3)
  - Anti-fibrinolytic (4)
  - Anti-inflammatory (5)
- But, CO…
  - Toxic gas (6)
  - Increases apoptosis (6)

1. Snyder SH, *et al.* 1998. *Brain Res Rev* 26:167-175.
2. Kourembanas S 2002. *Antioxid Redox Signal* 4:291-299.
3. Cardell LO, *et al.* 1998. *Pulm Pharmacol Ther* 11:309-315.
4. Fujita T, *et al.* 2001. *Nat Med* 7:598-604.
5. Otterbein LE, *et al.* 2000. *Nat Med* 6:422-428.
6. Clayton CE, *et al.* 2001. *Am J Physiol Lung Cell Mol Physiol* 281:L949-957.

What is protective about HO?

- **Bilirubin (formed from biliverdin via biliverdin reductase, slide 2)**
  - **Antioxidant (1,2)**
- **but…**
  - **There are significant toxicities of bilirubin (3,4)**

1. Stocker R, *et al.* 1987. *Science* 235:1043-1046.
2. Dennery PA, *et al.* 1995. *Free Radic Biol Med* 19:395-404.
3. Amato M, 1995. *Eur J Pediatr* 154:S54-59.
4. Amit Y, *et al.* 1989. *Pediatr Res* 25:364-368

What is protective about HO?

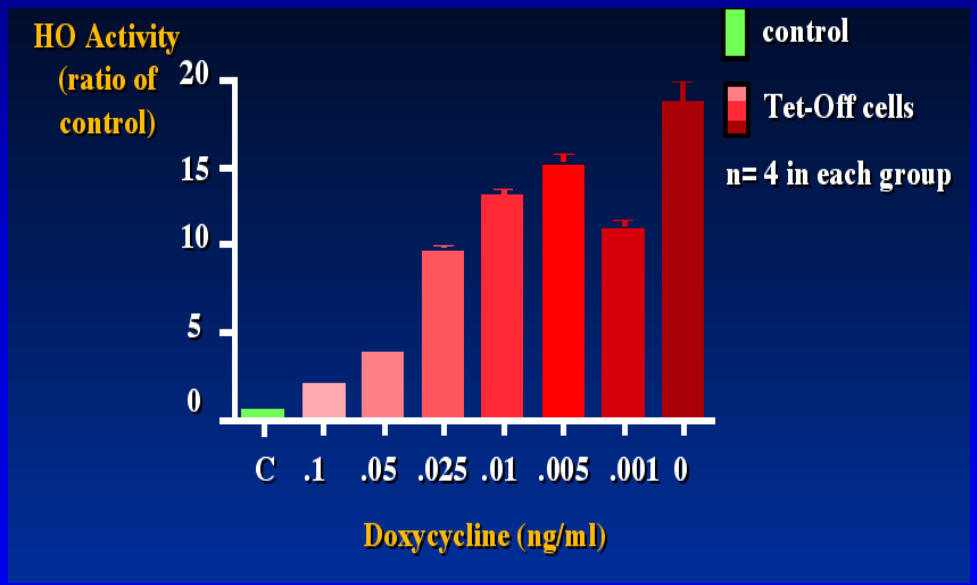
- **Sequestration of heme**
  - Removal of a pro-oxidant (1)
  - Co-induced ferritin sequesters heme iron released from the reaction (2)
- but…
  - Release of heme iron from the HO reaction (3)
  - Reactive iron generation in oxidant environment (3)
  - Ferritin is not always induced (4)

1. Balla G, *et al.* 1990. *Trans Assoc Am Physicians* 103:174-179
2. Balla G, *et al.* 1992. *J Biol Chem* 267:18148-18153
3. Suttner DM, Dennery PA 1999. *FASEB J* 13:1800-1808.
4. Ryan TP, *et al.* 1997. *Free Radic Biol Med* 22:901-908.

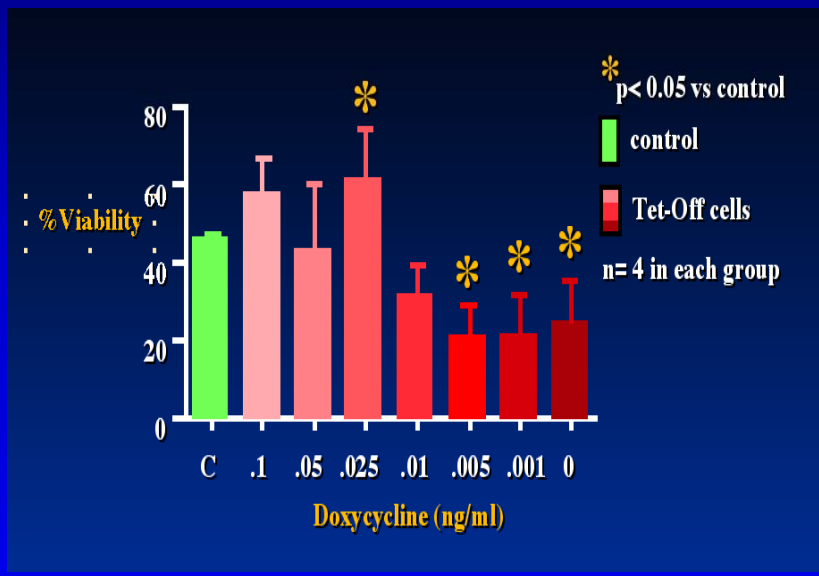
HO may not always be protective in the lung…

Example: After transfection of tetracycline regulatable HO-1 cDNA (A), protection against hyperoxia (increased viability (B), decreased lipid peroxidation and protein oxidation) was observed in the moderate range whereas detrimental effects occurred in the higher range of HO-1 overexpression (1).

A.



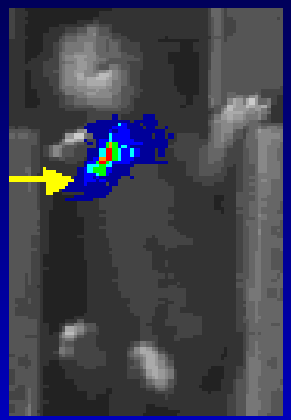
B.



1. Suttner, et al 1999, FASEB J. 13: 1800-08.

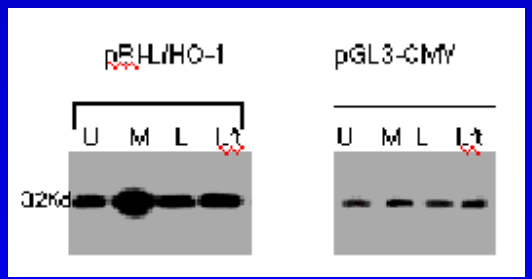
HO may not always be protective in the lung…

Example:

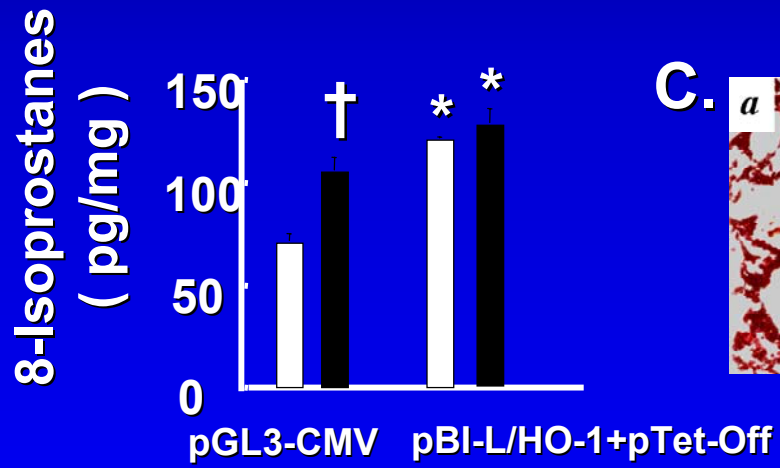


After intrathoracic HO-1 gene delivery into the right lung of neonatal mice (1):
-increased HO-1 gene expression (A)
- increased evidence of oxidative injury:
  -Increased 8-isoprostanes (B)
  -Increased iron deposition (C)

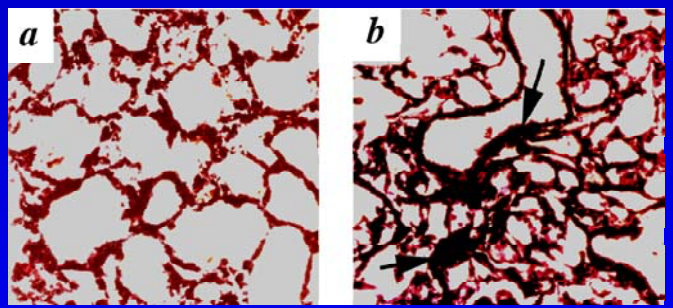
A.



B.



C.



1. Weng YH, *et al.* 2000, *Am J Physiol Lung Cell Mol Physiol* 278:L1273-1279.

But isn't HO-1 protective against hyperoxia in the lung ?

Yes:

- **Intratracheal delivery of HO-1 cDNA (1):**
  - Improved survival in hyperoxia
  - Decreased pulmonary edema

Primary target of HO-1 delivery: bronchiolar epithelium

1. Otterbein LE, *et al.* 1999. *J Clin Invest* 103:1047-1054.

But isn't HO-1 protective against hyperoxia in the lung ?

NO:

- Welty *et al.* : Transgenic mice with SP-C driven HO-1 over-expression (1):
  - Increased pulmonary edema

- Weng *et al.*:Transpulmonary HO-1 gene delivery (2):
  - Increased markers of oxidative injury
  - Increased iron deposition

Primary target of HO-1 delivery: Type II cells

1. Welty SE, *et al.* 1999. *Am Rev Respir Crit Care Med* 159:A218 (Abstract).
2. Weng YH, *et al.* 2000. *Am J Physiol Lung Cell Mol Physiol* 278:L1273-1279.

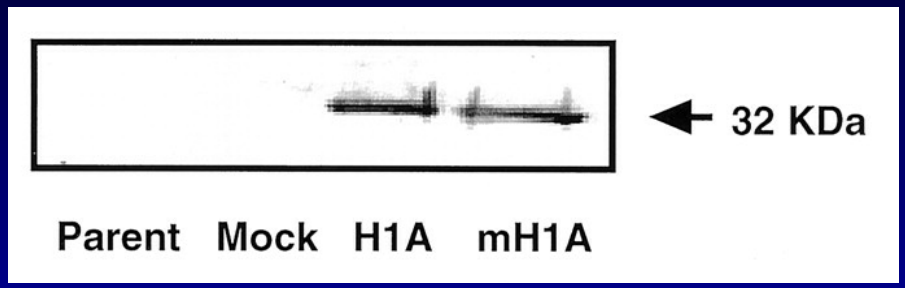
New thoughts about HO

- Heme oxygenase protein may have protective effects independently of its activity (1)
- HO protein may regulate other genes:
  - Catalase (2)
  - MnSOD (3)

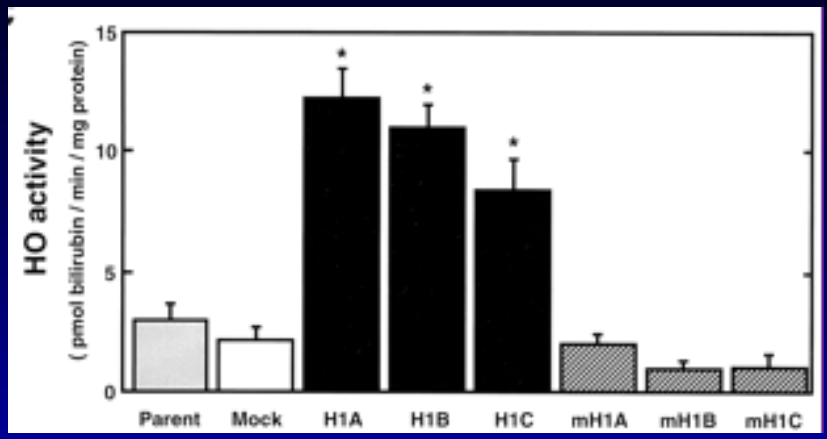
1. Taylor JL *et al.* 1998. *Am J Physiol* 274:L582-590.
2. Frankel D, *et al.* 2000. *J Cell Physiol* 185:80-86.
3. Hori R, *et al.* 2002. *J Biol Chem* 277:10712-10718.

Non-enzymatic effects of HO-1 protein

A.



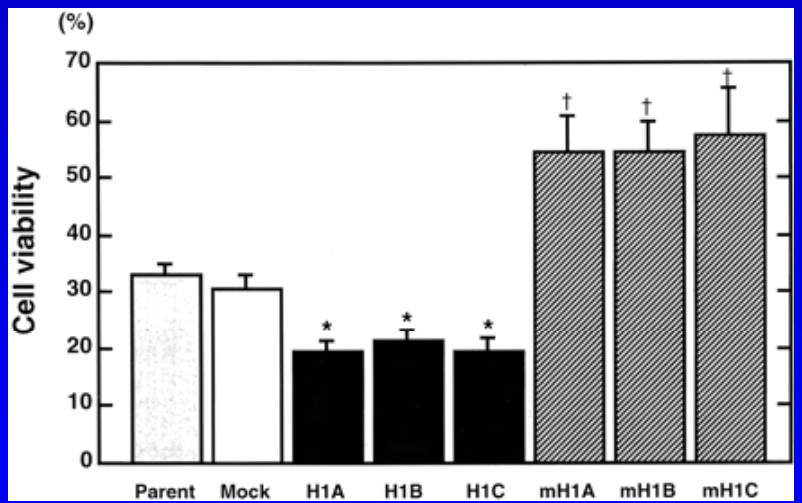
B.



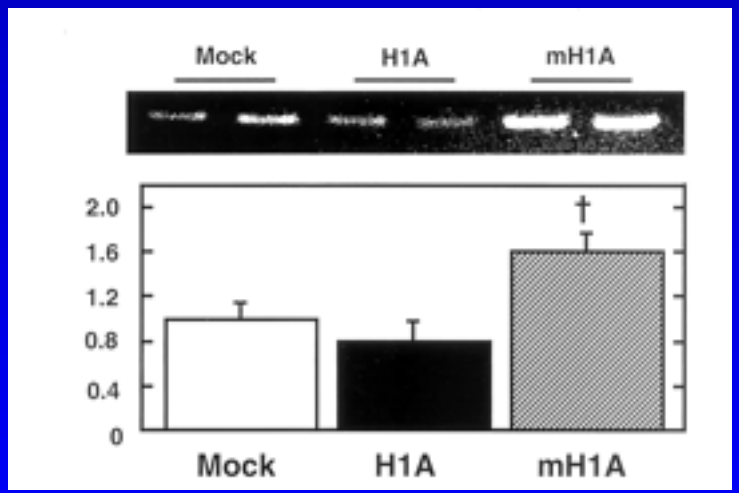
- After transfection of an active (H1A) and inactive (mH1A) strain of HO-1 (A,B), differences in cell viability in H_2O_2 (C) and catalase expression (D) were observed (1).

1. Hori *et al.* 2002. *J. Biol Chem*, 277:10712-10718.

C.



D.



Summary

- HO degrades heme to form bile pigments and CO.
- HO-1 is readily inducible in oxidative stress.
- Both HO-1 and HO-2 are found in the lung throughout development.
- Moderate expression of HO-1 is protective.
- HO-2 is also protective against oxidative injury.
- There are circumstances when HO is not protective.
- There may be non-enzymatic effects of HO-1 (*i.e.* effects of inactive HO-1 protein).