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**Developmental Regulation of Heme Oxygenase Expression in the Mouse Brain**

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Heme oxygenase is the rate-limiting enzyme in heme degradation pathway. There are two well-described isozymes, the inducible HO-1 and the constitutive, HO-2.<sup>1</sup> A third isozyme (HO-3) has been identified, but its role is incompletely known.<sup>2</sup> HO-2 is the predominant isozyme in the adult rodent brain.<sup>1</sup> For the study of HO-1 transcriptional regulation, we have developed a transgenic mouse model (HO-1-*luc* Tg), where the transgene is comprised of the full-length HO-1 promoter driving expression of a modified *luciferase* (*luc*) reporter gene.<sup>3,4</sup> This model allows us to noninvasively monitor, through bioluminescence imaging (BLI), any changes in HO-1 transcription through proportional changes in *luc* activity as quantitated by photons emitted per sec.<sup>3,5</sup> During the development of this mouse model, we observed that, in contrast to the adult mouse, the newborn mouse exhibited significant HO-1 gene expression in the brain. The objective of this study was to characterize the pattern of HO expression in the developing mouse brain through measurements of *in vivo* HO-1 transcription, HO-1 and HO-2 protein, and total HO enzyme activity levels.

HO-1 transcription levels were assessed in HO-1-*luc* Tg mice, ranging in age from 1 to 35 days, by BLI of the head region using a cooled charge-coupled device camera (IVIS™, Xenogen Corp., Alameda CA).<sup>3,5</sup> Brain tissue (n = 3 for each age group) was then harvested and sonicated with buffer. HO-1 and HO-2 proteins were separated by polyacrylamide gel electrophoresis, detected by Western blot analysis, and quantitated by densitometry. Results from these analyses are expressed as fold change from adult (35 day-old) levels. Sonicate HO activity, quantitated via measurements of carbon monoxide (CO) by gas chromatography,<sup>6</sup> is expressed as mean ± SD, pmol CO/h/mg fresh weight (FW). The results are shown on the Table below:

Age (in days)	1	3	7	14	21	28	35
<b>HO-1 Transcription</b>	20.4±13.4*	3.8±1.7	4.5±0.2	1.8±1.7	0.7±0.4	0.4±0.3	1.0
<b>HO-1 Protein</b>	10.2±1.4*	8.1±0.2*	6.0±0.6*	4.8±0.1*	1.8±1.9	1.1±0.2	1.0
<b>HO-2 Protein</b>	1.4±0.0	1.1±0.3	1.3±0.1	1.2±0.1	1.1±0.1	0.8±0.1	1.0
<b>HO Activity</b>	108±11*	55±8	71±4	90±12	96±4	87±6	80±11

\* $p \leq 0.05$  as compared to 35-day old levels

We found that HO-1 transcription, HO-1 protein, and HO enzyme activity levels are dramatically elevated at birth. These levels then decrease progressively during maturation to adulthood. HO-2 protein levels, as expected, remained fairly constant during all developmental stages.

We conclude that HO-1 expression in the mouse brain is not only high at birth, but it is also developmentally-regulated. This developmental pattern may be associated with tissue differentiation, cell population changes, loss of specific HO-1 inducers, or gene silencing.

References

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