

(12)

**LIPID PEROXIDATION-GENERATED CARBON MONOXIDE IN MICE:  
EFFECT OF VITAMIN E AND METALLOPORPHYRINS**

**Hendrik J. Vreman, Ronald J. Wong, Ganesh M. Shankar, David K. Stevenson**

Department of Pediatrics, Stanford University School of Medicine, Stanford, CA

Because carbon monoxide (CO), generated via heme degradation, has a role in the regulation of cellular functions,<sup>1</sup> non-enzymatic sources of CO, i.e., lipid peroxidation (LP), may also be important. We have shown that CO is a significant product of *in vitro* Fe<sup>2+</sup>/ascorbate-mediated oxidation of lipids in rat tissue membranes.<sup>2,3</sup> Brain, spinal cord, and kidney homogenates had 10X greater CO production rates than other major organs, which we determined were protected by cytoplasmic antioxidants. We hypothesized that cytoplasmic antioxidant levels were related to the amount of Vitamin E (Vit E) in the rodent feedstock (75 mg/kg).<sup>4,5</sup> The objective of our study was to determine: **A)** the native level of LP-mediated CO production from organ preparations of rodent chow-fed mice; **B)** if dietary Vit E removal decreases this CO production; **C)** if Vit E administration restores CO production; and **D)** if metalloporphyrin (Mp) inhibitors of HO affect LP *in vitro*.<sup>6,7</sup>

Adult male BALB/C mice, raised on regular rodent chow were used. For **A**, organs were harvested from chow-fed mice for the determination of native tissue LP-mediated CO generation rates. For **B**, mice were fed a Vit E-free diet and sacrificed for up to 7d for determination of the change in tissue CO generation rates. For **C**, mice were fed the Vit E-free diet for 7d and then injected IP with 15- $\mu$ g Vit E/g BW. For **D**, brain and kidney were harvested from chow-fed mice for determination of the Mp concentration required for 50% inhibition (I<sub>50</sub>) of LP-mediated CO production. 20% sonicates of all harvested organs were prepared in phosphate buffer. LP-mediated-CO generation rates were determined from 20- $\mu$ L tissue (4 mg) sonicate incubated with 6 $\mu$ M Fe<sup>2+</sup> and 100 $\mu$ M ascorbate in 60- $\mu$ L reaction mixtures for 30 min at 37°C in septum-sealed vials. Blank reactions also received 100 $\mu$ M BHT. For the I<sub>50</sub> determinations, metal-free (Mf), zinc (Zn), tin (Sn), and chromium (Cr) deuterio- (DP), proto- (PP), meso- (MP), and bis glycol (BG) porphyrins, were added in increasing concentrations into reaction mixtures with brain and kidney sonicates. Vial headspace CO was quantitated by gas chromatography and CO generation rates (mean  $\pm$  SD) expressed as pmol CO/h/mg fresh weight were as follows:

Treatment	Intestine	Liver	Kidney	Heart	Lung	Testes	Brain
<b>A) Chow-fed</b>	5 $\pm$ 3	7 $\pm$ 2	87 $\pm$ 18	100 $\pm$ 39	105 $\pm$ 38	118 $\pm$ 34	274 $\pm$ 57
<b>B) Vit E-free</b>	81 $\pm$ 18*	152 $\pm$ 28*	266 $\pm$ 45*	148 $\pm$ 24*	287 $\pm$ 26*	148 $\pm$ 7*	280 $\pm$ 47
<b>C) IP Vit E</b>	ND	1 $\pm$ 1	22 $\pm$ 6*	18 $\pm$ 22*	35 $\pm$ 28*	ND	201 $\pm$ 1

For **D**, the I<sub>50</sub>'s ( $\mu$ M) for brain and kidney sonicates, respectively, were: ZnMP (0.5, 1.0); ZnDP (1.0, 2.3); MfMP (8.5, 30.6); MfDP (12.2, >48); ZnPP (21.0, >48); CrMP (26.9, 28.8); SnBG (30.2, 35.0); and ZnBG (39.5, >48). I<sub>50</sub>'s of all other Mps were >48  $\mu$ M. \*p $\leq$ 0.05 from **A**.

We conclude that in chow-fed mice, brain, testes, lung, heart, and kidney produced significant LP-mediated CO due to relatively low antioxidant levels in these tissues. In contrast, liver and intestine appear to have high antioxidant levels, and thus produce low rates of CO. Removal of dietary Vit E increased CO production by all tissues, particularly the liver and intestine, but not brain, which appears to have reached its maximum rate. IP Vit E administration effectively restored CO production to chow-fed levels or below. Finally, Mp inhibitors of HO, also inhibit LP-mediated CO production, which suggests they may also act as antioxidants under some conditions.

In summary, the mouse, fed a Vit E-free diet, is a useful model for studying the efficacy of administered antioxidants and pro-oxidants, as well as the role of LP-generated CO in physiologic processes, such as vascular tone or neuronal function.

#### References

1. Marks GS, Brien JF, Nakatsu K, McLaughlin BE. Does carbon monoxide have a physiological function? *Trends Pharmacol Sci* 12:185-8, 1991.
2. Wolff DG, Bidlack WK. The formation of carbon monoxide during peroxidation of microsomal lipids. *Biochem Biophys Res Commun* 73:850-7, 1976.
3. Vreman HJ, Wong RJ, Sanesi CA, Dennery PA, Stevenson DK. Simultaneous production of carbon monoxide and thiobarbituric acid reactive substances in rat tissue preparations by an iron-ascorbate system. *Can J Physiol Pharmacol* 76:1057-65, 1998.
4. Kornbrust DJ, Mavis RD. Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: correlation with vitamin E content. *Lipids* 15:315-22, 1980.
5. Lehr HA, Vajkoczy P, Menger MD, Arfors KE. Do vitamin E supplements in diets for laboratory animals jeopardize findings in animal models of disease? *Free Radic Biol Med* 26:472-81, 1999.
6. Imai K, Aimoto T, Sato M, Kimura R. Antioxidative effect of several porphyrins on lipid peroxidation in rat liver homogenates. *Chem Pharm Bull (Tokyo)* 38:258-60, 1990.
7. Wong RJ, Vreman HJ, Stevenson DK. (Metallo)porphyrin inhibitors of heme oxygenase also inhibit lipid peroxidation (LP). *Pediatr Res* 47:79A, 2000.