

founding due to this factor. Our results are thus interpreted as the hazard ratio of recurrence for paravertebral *versus* general anesthesia for patients at the same histologic grade, and similarly for other factors in the model. This sort of multivariable analysis compensates for small, or even moderate, imbalances at baseline. We adjusted for this factor because of the retrospective nature of the study, even though we did not have evidence of it being a true confounder because it was not associated with the treatment groups ($P = 0.16$) or the outcome ($P = 0.25$), both of which are required by the classic definition of confounding.

As specified in the article, a single surgeon performed all cases in both groups. And again as specified, all paravertebral anesthesia was performed by a single anesthesiologist (D.J.B.), who also performed some of general anesthesia alone cases. The remainder were performed by three other attending anesthesiologists. The cases were similar, and the primary determinant of anesthetic type was assignment to D.J.B., who was the only anesthesiologist in the group familiar with the paravertebral technique.

The substantial limitations of observational studies are well known and were discussed in our article. For example, we specified: "Patients were not randomized and clinical care was not standardized, so that selection bias and the effects of unmeasured confounding variables cannot be excluded. For example, patients in the general anesthesia group had slightly larger tumors, smaller margins, and higher chemotherapy rates

than patients in the paravertebral group, factors that could affect mortality, although these differences did not reach statistical significance. Relevant information such as the amount of morphine given and the type of chemotherapy used in each group was not available in the records."

Under no circumstances should a small retrospective study be the basis for practice, and we suggested no such thing in our report. In contrast, the conclusion of our article was that "this study should be viewed as generating a hypothesis and an estimated effect size for future large randomized controlled trials, which are being planned and which will require several years for execution and analysis." A prospective trial is now in progress (ClinicalTrials.gov No. NCT00418457).

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Heme as a Playmaker in the Regulation of the Nitric Oxide System

To the Editor:—We read with great interest the article by Tsai *et al.*¹ In this article, the authors presented a laboratory investigation in which they showed that heme oxygenase-1 (HO-1) induction significantly inhibits type 2 cationic amino acid transporter expression and L-arginine transport in lipopolysaccharide-stimulated macrophages. The authors further suggested that this effect may be related to the activation of nuclear factor erythroid 2-related factor 2 and inhibition of nuclear factor κ B. After we read this analysis, it occurred to us that some points may be added to the discussion.

The authors showed that lipopolysaccharide treatment resulted in a significant increase in type 2 cationic amino acid transporter expression and this effect was reversed by concomitant treatment with heme (fig. 1). However, there are no data indicating the effect of heme treatment on nitric oxide formation. These set of experiments could have rendered the authors' conclusions stronger; in fact, heme may act as a pro-oxidant molecule, thus leading to an increased expression of the inducible isoform of nitric oxide synthase, which in turn leads to increased nitric oxide production. In this case, heme, although resulting in a significant decrease in type 2 cationic amino acid transporter expression and activity, may still induce the release of nitric oxide. In addition, heme serves as prosthetic group of inducible isoform of nitric oxide synthase, and thus heme treatment may result in an increased synthesis of the enzyme. Different HO-1 inducers, such as SnCl₂ or cobalt-protoporphyrin, could have added more information because they potentially induce HO-1 without increasing intracellular heme levels. In this regard, we and other authors previously showed that HO-1 induction by using cobalt-protoporphyrin or gene targeting modulates intracellular heme level, thus regulating the synthesis of heme-dependent proteins such as nitric oxide synthases, cyclooxygenases, nicotinamide adenine dinucleotide phosphate oxidase, and cytochrome P-450.^{2,3} These observations may be consistent with previous work performed by the same authors⁴ showing that propofol treatment resulted in a concomitant reduction of both the inducible isoform of nitric oxide synthase and type 2 cationic amino acid transporter expression. In this regard, we also showed that propofol may act as an inducer of HO-1 *via* activation of the nuclear factor- κ B pathway.⁵ Another point that we believe needs to be raised is in regard to the

authors' choice of adding heme immediately after lipopolysaccharide stimulation, thus not permitting a strong preinduction of HO-1 activity, which would have allowed increased carbon monoxide levels and a reduction of the intracellular heme pool. Interestingly, the authors also showed that tin protoporphyrin, a strong inhibitor of HO activity, results in a significant increase of HO-1 protein (even though in the Results section it was indicated that tin protoporphyrin did not increase protein expression) and partial reversion of heme effects. The molecular mechanism underlying this effect is still unclear, and several hypotheses may be carried out. One is that HO activity inhibition after tin protoporphyrin treatment results in increased intracellular heme level after strong HO activity inhibition, thus leading to increased HO-1

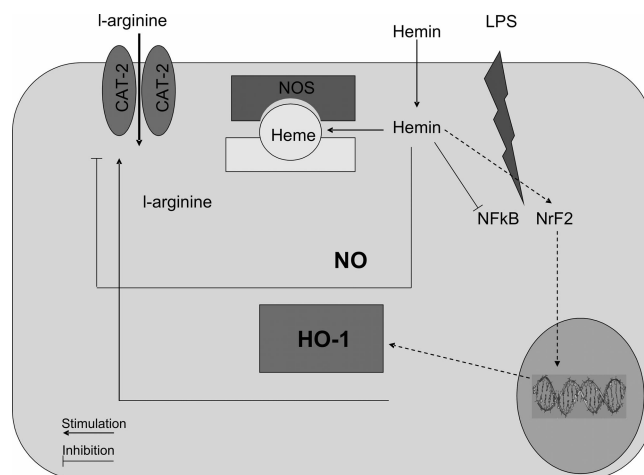


Fig. 1. Schematic representation of possible mechanisms involved in the interaction between heme and the nitric oxide system. CAT-2 = type 2 cationic amino acid transporter; HO-1 = heme oxygenase 1; LPS = lipopolysaccharide; NF κ B = nuclear factor κ B; NOS = nitric oxide synthase; Nrf2 = nuclear factor erythroid 2-related factor 2.

expression. The second hypothesis is based on the fact that because tin protoporphyrin possesses a structure similar to that of heme, it may directly induce HO-1 expression, probably by binding to specific heme binding motifs and activating the gene transcription. Moreover, the authors' observations further confirm that the activity of the protein rather than its expression or intracellular localization is required for its beneficial effects. We also agree with the authors regarding their conclusions about carbon monoxide. In fact, further studies using carbon monoxide-releasing molecules or carbon monoxide gas are required to confirm a possible interaction between carbon monoxide, nuclear factor erythroid 2-related factor 2, and nuclear factor κ B under these experimental conditions. In this regard, further studies should also be performed to exclude a possible involvement of biliverdin, the other byproduct of HO activity, which has also been shown to impact on inducible nitric oxide synthase expression and activity.^{6,7}

In conclusion, the authors' observations are novel and intriguing and add a new, important piece in the complicated puzzle of the interaction between the HO-carbon monoxide and nitric oxide-nitric oxide synthase systems.

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In Reply:—We appreciate the interest of Li Volti *et al.* in our recently published article.¹ In their letter, Li Volti *et al.* pointed out that our conclusions could have been strengthened if there had been data indicating the effect of hemin on nitric oxide. Incidentally, we did perform these experiments, but we decided not to present these data in the article because of page limitations. We appreciate these comments and are glad to present these data in this response letter. Briefly, production of nitric oxide and expression of inducible nitric oxide synthase, *i.e.*, the main enzyme for nitric oxide production during sepsis,² were evaluated by chemiluminescence and immunoblotting assays, as we previously reported.³ The data revealed that hemin significantly attenuated inducible nitric oxide synthase expression and nitric oxide production in lipopolysaccharide-stimulated macrophages (fig. 1). In addition, the effects of hemin on inducible nitric oxide synthase expression could be attenuated by tin protoporphyrin, the potent heme oxygenase-1 inhibitor (fig. 1). Along with our previous reports,^{4,5} these data provide strong evidence to support the crucial role of heme oxygenase 1 on regulating inducible nitric oxide synthase expression and nitric oxide production during sepsis.

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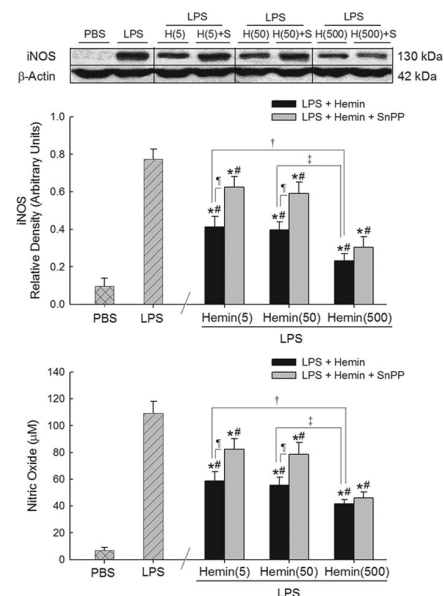


Fig. 1. Representative gel photograph illustrated the effects of hemin on inducible nitric oxide synthase (iNOS) expression and nitric oxide production in lipopolysaccharide-stimulated murine macrophages. The effects of tin protoporphyrin (SnPP) on iNOS expression and nitric oxide production in lipopolysaccharide (LPS) plus hemin-stimulated murine macrophages were also illustrated. The iNOS protein concentrations were determined using chemiluminescence assay. Data are expressed as mean \pm SD. H(5) and Hemin(5) = 5 μ M hemin; H(50) and Hemin(50) = 50 μ M hemin; H(500) and Hemin(500) = 500 μ M hemin; PBS = phosphate-buffered saline; S = tin protoporphyrin. * $P < 0.05$ compared with the PBS group. # $P < 0.05$ compared with the LPS group. † $P < 0.05$, the LPS + Hemin(500) group *versus* the LPS + Hemin(5) group. ‡ $P < 0.05$, the LPS + Hemin(500) group *versus* the LPS + Hemin(50) group. § $P < 0.05$, the LPS + Hemin(5) group *versus* the LPS + Hemin(5) + SnPP group or the LPS + Hemin(50) group *versus* the LPS + Hemin(50) + SnPP group.