

Reply

To the Editor:

Inoue et al.¹ raise important questions regarding our recent publication showing that juglone, a naphthoquinone inhibitor of the prolyl isomerase Pin1, suppresses eosinophilic pulmonary inflammation.² They recently reported that 1, 2-naphthoquinone found in diesel exhaust exacerbates allergy and pulmonary inflammation in mice.³ Juglone is found in the leaves, roots, and bark of plants in the Juglandaceae family, particularly the black walnut. Because of its dark orange-brown color, juglone has found use as a dye in the food and cosmetics industry, where it is known as C.I. Natural Brown 7 or C.I. 75500. Juglone toxicity is modest, with a median lethal dose of 112 mg/kg after oral administration in rats.⁴ Mice tolerated 10-fold more juglone administered intraperitoneally than orally.^{4,5} There are no published toxicity data after aerosol administration of juglone. Rats injected for 2 weeks intraperitoneally with juglone (1 mg/kg/d) showed normal renal, hematologic, and liver function.⁶ Pulmonary function measures (forced vital capacity and FEV_{0.2}) were normal in rats after 3 daily intraperitoneal juglone doses, and bronchoalveolar lavage fluid cell numbers and differential counts were unchanged from control values (L. A. Rosenthal, R. L. Sorkness, and J. S. Malter, unpublished data, 2007). However, consistent with Inoue et al.,³ we found that pulmonary insufflation of 1 mg/kg juglone dissolved in surfactant was rapidly lethal because of fluid accumulation in the lungs (L. A. Rosenthal, S. Esnault, and J. S. Malter, unpublished data, 2006). Therefore naphthoquinone toxicity varies greatly depending on the route of delivery and concentration in lung tissue.

The focus of our previous report² was that Pin1 facilitated allergic eosinophilic pulmonary inflammation, likely through the regulation of GM-CSF and IL-5 production and pro-survival signaling. We have observed equivalent results on eosinophil survival and cytokine expression after *in vitro* transduction with TAT-linked, dominant-negative Pin1 peptides^{2,7} and on allergic lung fibrosis *in vivo* in allergen-sensitized and allergen-challenged Pin1^{-/-} mice.⁸ Therefore we are confident that Pin1 plays a key role in eosinophilic pulmonary inflammation and might well be a promising therapeutic target for asthma and other pulmonary diseases. The development of specific and selective Pin1 antagonists is clearly necessary, and the cautionary note raised by Inoue et al.¹ and our unpublished work suggests naphthoquinone-based drugs might not be sufficiently safe for clinical use.

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REFERENCES

- Inoue K, Takano H, Kumagai Y. Pin1 blockade in asthma by naphthoquinone? *J Allergy Clin Immunol* 2008;121:1064.

- Esnault S, Rosenthal LA, Shen ZJ, Sedgwick JB, Szakaly RJ, Sorkness RL. A critical role for Pin1 in allergic pulmonary eosinophilia in rats. *J Allergy Clin Immunol* 2007;120:1082-8.
- Inoue K, Takano H, Hiyoshi K, Ichinose T, Sadakane K, Yanagisawa R, et al. Naphthoquinone enhances antigen-related airway inflammation in mice. *Eur Respir J* 2007;29:259-67.
- NLM (1999a) RTECS (Registry of Toxic Effects of Chemical Substances). Bethesda (MD): National Library of Medicine; searched May 1999. Record nos. 51579, 52239, 52274, 52282.
- Westfall BA, Russell RL, Auyong TK. Depressant agent from walnut hulls. *Science* 1961;134:1617.
- Esnault S, Braun RK, Shen ZJ, Xiang Z, Heninger E, Love RB, et al. Pin1 modulates the type 1 immune response. *PLoS ONE* 2007;2:e226.
- Shen ZJ, Esnault S, Malter JS. The peptidyl-prolyl isomerase Pin1 regulates the stability of granulocyte-macrophage colony-stimulating factor mRNA in activated eosinophils. *Nat Immunol* 2005;6:1280-7.
- Shen ZJ, Esnault S, Rosenthal LA, Szakaly RJ, Sorkness RL, Westmark PR, et al. Pin1 regulates eosinophil TGF- β 1 production by activated human and murine eosinophils and contributes to allergic lung fibrosis. *J Clin Invest* 2008;118:479-90.

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Allergy risk of vitamin D supplements has been described in various settings

To the Editor:

Litonjua and Weiss¹ highlight again the importance of vitamin supplements and allergic sensitization, although in a somewhat unexpected manner back to front of the original vitamin hypothesis.² Although describing correctly the T_H1 blocking activity of the main vitamin D metabolite 1,25-(OH)₂-D₃, they most likely misinterpret the recent increase of oral vitamin D supplementation that clearly follows "Western lifestyle" patterns. The vitamin hypothesis is not about solar exposure (which might indeed be low in some American communities) but about oral supplementation that might influence allergen presentation at the largest immunologic site of the body.

Certainly misquoted has been the study of Finnish newborns³ that described a positive (and not negative) association of regular vitamin D supplements and allergy. This study has been particularly important because the effect had been predicted in advance.² Together with other studies not mentioned in their review that are testing effects of different vitamin D preparations,^{4,5} cod liver oil,⁶ and supplemented margarine,⁷ the current picture of a sensitization risk caused by vitamin D supplements is rather clear. Even their own study results¹ are not contradictory because a reduced wheezing rate is an already expected effect of vitamin D supplementation.

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REFERENCES

- Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? *J Allergy Clin Immunol* 2007;120:1031-5.
- Wjst M, Dold S. Genes, factor X, and allergens: what causes allergic diseases? *Allergy* 1999;54:757-9.
- Hyppönen E, Sovio U, Wjst M, Patel S, Pekkanen J, Hartikainen AL, et al. Vitamin D supplementation in infancy and the risk of allergies in adulthood: a birth cohort study. *Ann Am Acad Sci* 2004;1037:84-95.
- Milner JD, Stein DM, McCarter R, Moon RY. Early infant multivitamin supplementation is associated with increased risk for food allergy and asthma. *Pediatrics* 2004;114:27-32.
- Kull I, Bergström A, Melén E, Lilja G, van Hage M, Pershagen G, et al. Early-life supplementation of vitamins A and D, in water-soluble form or in