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Letter to Editor

Rebuttal Letter

George A. Heavner[1]

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Dear Editor,

Hermeling *et al.* (1) continue to suggest that micelle-associated epoetin is still a possible explanation for the increase in PRCA that was seen related to the subcutaneous administration of EPREX® to patients with chronic renal failure. The researchers hold to this theory despite the fact that both clinical data and laboratory studies link the transient increase to an adjuvant effect of aromatic small molecules leached from the uncoated rubber stoppers by the polysorbate 80 introduced into the formulation in 1998 (3). The incidence rate of pure red cell aplasia (PRCA) with the polysorbate 80 formulation in prefilled syringes fitted with uncoated rubber stopper plungers was 4.61/10,000 patient-years of exposure (2), whereas the incidence rate for the same formulation in vials with a Teflon-clad closure is 0.36/10,000 patient-years of exposure. The incidence rate of the same formulation in prefilled syringes fitted with coated rubber stopper plungers is 0.26/10,000 patient-years of exposure. This rate is comparable with the rate for the human serum albumin (HSA)-containing formulation of 0.47/10,000 patient-years of exposure. Clearly, the remediation by replacement of the uncoated rubber syringe stoppers with coated rubber syringe stoppers directly addressed the root cause of PRCA without formulation changes.

Hermeling *et al.* (1) also discount the leachates as the causative factor of PRCA because "the leachates also failed to enhance the immune response against epoetin. Instead, their claim is based on a single experiment using an irrelevant antigen (ovalbumin, a foreign antigen, which is intrinsically immunogenic in mice) in combination with irrelevant leachate concentrations." These statements are incorrect. Studies of the leachates in a murine model in multiple experiments have demonstrated that they function as a weak adjuvant, generating antierythropoietin (anti-EPO) antibodies and decreasing hematocrit (3,4). The use of ovalbumin as a surrogate antigen was suggested by an immunology advisory board of internationally recognized immunologists that were convened on three different occasions to provide expert evaluation and advice in the investigation into the cause of PRCA.(2) It was the consensus of this advisory board that testing the adjuvant properties of the leachates in the mouse ovalbumin model was a more rigorous study as it is a well-defined model for this type of study. The leachates demonstrated a dose-dependent adjuvant effect where the amount of leachates dosed ranged from the equivalent amount found in from 2 to 32 syringes. When compared to ovalbumin alone, a statistically significant increase in the immune response to ovalbumin was observed even at the lowest dose of leachates administered.

The Hermeling group questions the relevance of studies done with concentrations of polysorbate 80 higher than that found in EPREX®. If their hypothesis of erythropoietin associating with micelles is correct then one would expect that in studies with higher concentrations of polysorbate 80, the amount of protein found in the polysorbate 80 peaks in the size-exclusion chromatography (SEC)/high-performance liquid chromatography (HPLC) would increase in proportion to the polysorbate 80 concentration. At concentrations up to ten times that present in EPREX®, we have not observed any increase in protein coeluting with the polysorbate 80. This is consistent with the protein coeluting with polysorbate 80 being dimer/oligomer and not micelle-associated protein. Our studies have included materials that were freshly prepared and EPREX® that was more than 20 months old with no differences in results. Indeed, in several lots of prefilled syringes that were more than 20 months old, ELISA assays could detect no material coeluting with the polysorbate 80 peaks.

To our knowledge the Hermeling group has not been able to provide any experimental evidence that would further support their claim beyond the SEC data. Because it is an experimental fact that EPO dimers and oligomers elute on an SEC column in the same region as polysorbate 80, it is scientifically inappropriate for these authors to continue to make claims that their data indicate the existence of a complex micelle–EPO interaction without providing significant corroborative evidence.

REFERENCES

1. S. Hermeling, H. Schellekens, D. J. A. Crommelin, and W. Jiskoot. Micelle-associated protein in epoetin formulations: a risk factor for immunogenicity? *Pharm. Res.* **20**:1903–1907 (2003).

[1] Centocor, Horsham, Pennsylvania, USA.

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2. K. Boven, S. Stryker, J. Knight, A. Thomas, M. van Regenmortel, D. M. Kemeny, D. Power, J. Rossert, and N. Casadevall. The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes. *Kidney Int.* **67**:2346–2353 (2005).
3. B. Sharma, F. Bader, T. Templeman, P. Lisi, M. Ryan, and G. A. Heavner. Technical investigations into the cause of the increased incidence of antibody-mediated pure red cell aplasia associated with Eprex®. *Eur. J. Hosp. Pharm.* **5**:86–91 (2004).
4. M. H. Ryan, G. A. Heavner, M. Brigham-Burke, F. McMahon, M. F. Shanahan, S. R. Gunturi, B. Sharma, and F. X. Farrell. An *in vivo* model to assess factors that may stimulate the generation of an immune reaction to erythropoietin. *Int. Immunopharmacol.* **6**:647–655 (2006).