Letters to the Editor

Differences in efficacy found in animals between recombinant forms of erythropoietin will not necessarily translate into differences in humans

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Sir

In your supplemental issue for volume 84, dated April 2001, Egrie and Browne (2001) reported a compilation of studies on the characterisation of novel erythropoiesis stimulating protein (NESP). NESP is a mutated form of the native hormone, erythropoietin. In NESP, five amino acids have been mutated (i.e., Ala30Asn, His 32Thr, Pro87Val, Trp88Asn and Pro90Thr) in order to incorporate two additional N-linked carbohydrate chains for a total of five carbohydrate moieties. In contrast, human erythropoietin as well as its recombinant form, r-HuEPO, has three N-linked carbohydrate chains. For comparative studies with NESP, Egrie and Browne (2001) used r-HuEPO.

In this report, it is shown that by increasing the sialic acid residue content of erythropoietin, the serum half life of the recombinant molecule, NESP, was approximately three-fold longer than that of r-HuEPO, primarily due to a prolonged clearance from the circulation. However, features in common between the two molecules are their nonlinear pharmacokinetics and pharmacodynamics. An increase in dose is required for both molecules when switching from a thrice-weekly (TIW) to a once-weekly (QW) regimen. For example, NESP $1.25 \mu\text{g kg}^{-1}$ TIW (total weekly dose: $3.75 \mu\text{g kg}^{-1}$) had to be increased four-fold to $15 \mu\text{g kg}^{-1}$ QW in order to achieve a nearly identical biological response. For r-HuEPO, the authors claimed that a 15-fold increase in the weekly dose of r-HuEPO was needed to switch from the TIW to the QW regimen in the animal model tested. In light of these findings, the authors continue to state that NESP has a 'greater' efficiency than r-HuEPO due to its 'greater' potency. Since pharmacodynamic responses depend on dose, route of administration, and dosing regimen, it is apparent that additional experimental data would be needed to substantiate a statement regarding the 'greater' potency of NESP. In the animal model tested, the data presented were derived from doses administered intravenously, and no comparison was made with alternative delivery routes (e.g., subcutaneous). Moreover, these results in animals appear to be of no consequence, since in humans, a similar biological response for NESP was achieved with an optimal weekly dose administered either once or divided into three doses (Macdougall, 1998). Similarly for r-HuEPO, 150 IU kg^{-1} TIW (total weekly dose: 450 IU kg^{-1}) and 600 IU kg^{-1} QW (approximately

40 000 IU QW) regimens have been shown to produce a similar pharmacodynamic response in the rise of haemoglobin (Cheung *et al*, 1998). Thus, with the major differences in the erythropoiesis and red blood cell lifespan between rodents and humans, one must be cautious in extrapolating the data of *in vivo* biologic activity based upon small animal models to humans.

It is also important to note that potency cannot be equated with efficacy. In another set of experiments, efficacy was investigated using equimolar doses (i.e., equivalent peptide mass) of r-HuEPO, an erythropoietin analogue containing four N-linked carbohydrates, and NESP. For example, the results of one of these experiments showed that the haematocrit in mice increased by 22.8 ± 2.3 , 28.1 ± 6.8 , and 33.9 ± 3.4 points, respectively. The authors concluded that since the bulkiness of NESP lowers its affinity for the erythropoietin receptor, clearance (i.e., *in vivo* potency) was the primary determinant for the higher magnitude in efficacy. With due respect to the authors, your readership is misled by the assessment that NESP has an enhanced efficacy compared with r-HuEPO or the four N-linked chain analogue. It appears that the authors are remiss in not considering that an equimolar dose is not equivalent to an equipotent dose, and thus conclusions regarding efficacy cannot be made. The use of equipotent doses would have been the appropriate experimental design for testing efficacy.

NESP is a product of only five point mutations, and the readership should be aware that some of the point mutations introduced into the erythropoietin molecule for the consensus sequences of additional carbohydrate moieties are nonconservative, especially those at residues 87, 88, and 90. In the native molecule, these residues contain ring structures. Since the predicted structure of endogenous erythropoietin is comprised of four α helices with a common secondary structural motif and bundled into a tertiary fold (Wrighton *et al*, 1996), these three bulky and more rigid residues potentially promote major turns in the polypeptide backbone. Thus in the NESP molecule, replacing these bulky residues with smaller and/or less rigid amino acids, the structure may change not only with additional carbohydrate moieties but also with the structural motif of the polypeptide backbone. As noted by the authors, the carbohydrate moieties may mask the point mutations themselves from the immune system; however, changes in secondary structure downstream from the point mutations cannot be ruled out.

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To date, human studies in a dialysis population have confirmed the pharmacokinetic differences in half life and clearance between NESP and r-HuEPO (Macdougall, 1999). However, studies in humans using NESP have shown an efficacy profile that is compar-

able to r-HuEPO (Coyne *et al*, 2000; Nissenson *et al*, 2000; Locatelli *et al*, 2001). These studies reinforce the fact that results garnered from animal models are not necessarily indicative of what is to be ascertained in humans.

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Darbepoetin alfa is more potent *in vivo* and can be administered less frequently than rHuEPO

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Sir

The development of darbepoetin alfa (NESP, ARANESPTM) was an outgrowth of basic research directed towards elucidating those structural features that control the *in vivo* biological activity of erythropoietin (EPO). As described in our study, this research demonstrated that serum clearance is the primary determinant of EPO *in vivo* biological activity, and that serum clearance can be manipulated by changing the proportion of sialic acid-containing carbohydrate. Molecules having a higher proportion of sialic acid-containing carbohydrate have a longer serum half-life and thereby a higher *in vivo* biological activity. These principles, gleaned from natural sequence EPO, were applied and extended to specifically engineer a new molecule, NESP. Our review article (Egrie and Browne, 2001) summarized the basic research leading to the design of NESP and summarized the results of comparative pharmacodynamic studies of NESP and rHuEPO.

As we stated in our article, the relative potency of NESP and rHuEPO were determined in a normal mouse animal model in which both molecules produced dose-dependent increases in hematocrit. These studies were performed using three different routes of administration and three different dosing frequencies. For each route, frequency, and test article, multiple doses were tested which covered the entire dose response range. The results of these experiments were used to construct relative potency plots (log dose response curves). The relative potency was then determined as the ratio of equieffective doses of each from their respective graded dose–response relations (Tallarida and Murray, 1987). When data from all studies were combined (nine experiments using 1185 animals), NESP was determined to be 3.6-fold more potent than rHuEPO for each route of administration when given three times per week. That is, it takes 3.6-fold more rHuEPO to obtain the same biological response as NESP. As a corollary, when equimolar doses of NESP and rHuEPO are compared, rHuEPO produces a lower biological response, as is illustrated in our Figure 5 and noted in paragraph 3 of Dr

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