

Extrapolation to human subjects

From the viewpoint of future clinical applications, predictions of human pharmacokinetics based on data obtained from animal studies—so called, “animal scale-up”—is important for the determination of optimal doses and intervals (Izumi et al., 1996). Thus, we attempted to predict the half-life of HbV in humans using an allometric equation that is generally used in animal scale-up studies. Using the relationships observed for mice (Taguchi et al., 2009b), rats (Taguchi et al., 2009a), and rabbits (Sou et al., 2005), the half-life of HbV in healthy humans was predicted to be approximately 96 hours. In addition, based on half-life data and percent of injected dose values obtained from pharmacokinetic studies of HbV in rats and rabbits, Sou et al. also predicted that the half-life of HbV in healthy humans would be approximately 72 hours (Sou et al., 2005). Further, the half-life of liposomal preparations is empirically 2–3-fold greater in humans than in rats (Gabizon et al., 2003). In fact, the half-life of liposomal doxorubicin (Doxil formulation) in rats and humans is 35 and 56–90 hours, respectively (Gabizon et al., 2003). Therefore, the half-life of HbV in humans would be predicted to be 3–4 days (Sou et al., 2005). For HbV to function as an artificial oxygen carrier, it is desirable that intravascular persistence be at least equal to the time required to regenerate RBCs (Sehgal et al., 1984). Following a massive hemorrhage, the lost blood volume and oxygen-carrying capacity is replaced within approximately 5 days (Hughes et al., 1995; Awasthi et al., 2007). Because the half-life of HbV in humans was estimated to be approximately 3–4 days, HbV would function as a temporary oxygen carrier until a blood transfusion is available or until autologous blood is recovered after a massive hemorrhage.

Conclusion

Like other drugs, a pharmacokinetic evaluation is an important issue for the development of HbV as a substitute of RBC. In fact, though the perfluorocarbon-based oxygen carriers and acellular-type HBOCs were moved into the clinical trial stages, these artificial oxygen carriers dropped from further clinical development due to severe and unexpected side effects, which might have been predicted from pharmacokinetic analysis data. Therefore, it is also necessary to conduct an in-depth pharmacokinetic study of HbV before moving on to the clinical trial stage.

Our recent preclinical study of HbV clearly demonstrated five major findings on pharmacokinetic profiles. First, HbV and its components have favorable metabolic and excretion profiles in mammalian species, similar to endogenous substances. Second, HbV is safe and useful under conditions of a massive hemorrhage. Third, HbV did not show any toxicity and accumulation in the body, even under conditions of hypometabolism and excretion (i.e., hepatic cirrhosis). Fourth, HbV has the potential to

induce the ABC phenomenon, but the repeated use of HbV at a putative dose would not be expected to induce the ABC phenomenon in a clinical situation. Finally, HbV has a good retention in the blood circulation, and the half-life of HbV in humans was estimated to be approximately 3–4 days, which is sufficient for it to function as an oxygen carrier. These findings support previous views related to the pharmacological efficacy and safety of HbV in normal and hemorrhagic shock model rats from the view point of pharmacokinetics.

In addition to functioning as a substitute for RBCs, HbV would be expected to have a variety of other applications, based on its oxygen transport characteristics, such as in cardiopulmonary bypass priming solutions (Yamazaki et al., 2006), wound healing in critically ischemic skin (Plock et al., 2009), and as a radiation therapy agent (Yamamoto et al., 2009). Therefore, this issue deserves to be studied further, with further data collected in preclinical pharmacokinetic studies for future applications of HbV in the clinic.

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Declaration of interest

The authors declare no financial conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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What Is the Major Mechanism of Slower NO Uptake by Red Blood Cells?

Azarov *et al.* (1), using our experimental data (2), resimulated the mechanism of slower NO uptake by RBCs and hemoglobin (Hb)-encapsulated vesicles. We inferred the contribution of the intracellular diffusion barrier and the absence of the membrane barrier. Although acknowledging an intracellular diffusion barrier, they insisted on the presence of a membrane barrier and a major contribution of an extracellular diffusion barrier. That presents some problems.

First, the membrane permeability of NO they used, $9 \times 10^5 \mu\text{m s}^{-1}$, originated from Subczynski *et al.* (3), who clarified that the permeability is equivalent to or greater than that of a water layer of equal thickness. This supports the absence of a membrane barrier.

Second, the diffusion constant of NO they used, $3300 \mu\text{m}^2 \text{s}^{-1}$ (37 °C) (4), is much higher than that in other papers (2, 3) and our intracellular diffusion constants (25 °C), which they used for simulation (2). The positional relation between a nanoparticle and an extracellular phase is fixed, in spite of the rapid motion of nanoparticles by the stopped-

flow method. Accordingly, they might overestimate the extracellular barrier.

Third, they suggest a cytoskeleton barrier. However, it is difficult to imagine the existence of more NO-binding and -reacting sites in a thin cytoskeletal layer than the abundant sites of the adjacent intracellular Hb solution. To measure the NO-binding rate constant, they used the gentle "competition method," which presents the possibility of slow motion and sedimentation of RBCs. Therefore, the extracellular and membrane barriers might be overestimated.

Their simulation requires corrections to improve its reliability.

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Reply to Sakai: What Is the Major Mechanism of Slower NO Uptake by Red Blood Cells

This is a response to a letter by Hiromi Sakai (1)

In their original work Sakai and colleagues (2) suggested that internal diffusion alone can account for slower NO uptake by hemoglobin-containing vesicles. Our simulations are consistent with this conclusion, but we show that better agreement between experiment and theory is obtained when all three mechanisms (internal diffusion, external diffusion, and membrane permeability) are included. Moreover, our simulations show that for phospholipid vesicles and red blood cells, external diffusion has the largest effect. Importantly, our experimental data showing that viscosity of the external medium affects NO uptake by red cells provide unequivocal evidence for a role of external diffusion, contrary to the position held by Sakai. This same approach, combining experimental and theoretical data, showed that external diffusion is the main factor limiting oxygen uptake by red cells 30 years ago, and one would not expect a big difference with NO (3, 4).

We have always held that membrane permeability plays a small role in limiting NO uptake compared to other factors in most cases. However, in the case of red cell microparticles, we find that it plays a major role. It should be remembered that these microparticles only take up NO a few times slower than free hemoglobin (whereas red cells take up NO up to 1000 times slower), so the effect is still small. Like Professor Liao (5, 6), we suggest the effects on membrane permeability are due to the protein scaffold, and this notion is strongly supported by data in which the protein scaffold was physically or chemically modified (6).

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