DISCUSSION

The three rHuEPO preparations; Epokine®, Eprex®, and Recormon® are biological medicinal products containing biotechnology-derived recombinant erythropoietin as an active substance. The three products contain a similar amino acid sequence as endogenous erythropoietin but differ in their glycosylation patterns which influence their pharmacokinetics and may affect their efficacy and safety (68, 69, 70). In general, EPO beta (Recormon®) has a higher molecular weight, contains a wider spectrum of isoforms with a higher proportion of more basic isoforms, consistent with a lower number of sialylated glycan residues than EPO alpha (Eprex®). The differences in their structures resulted in a larger volume of distribution, a longer elimination half-life and a more delay in drug absorption after subcutaneous administration of EPO beta when compared to EPO alpha (70). Epokine® is a new recombinant erythropoietin claimed to be similar to EPO alpha product. The clinical studies on the safety and efficacy of Epokine® for the treatment of anemia in patients with end stage renal disease and diabetic nephropathy have been previously reported in a small number of subject (31). Nonetheless, due to a complexity of a biotechnology manufacturing process, the structure of this new biological product may differ from the innovator. In order to demonstrate the similarity of the new EPO preparation, the pharmacokinetic study should be determined before further study on pharmacodynamic as well as clinical efficacy and safety studies.

The baseline endogenous erythropoietin concentrations obtained at 9 PM from this study were averaged from 6-7 mIU/mL with a ranged of 3.5–11.4 mIU/mL. These values were within the normal values of 3.3-32.0 mIU/mL reported in healthy subjects (1, 8, 9). The basal morning erythropoietin concentrations in this study could be used as a normal value for healthy male however could not represent the erythropoietin concentration at various times since the erythropoietin levels varied during a 24 h as diurnal variation.

The pharmacokinetic assessment of the three biological products after subcutaneous administrations at the upper arm showed the slow absorption rate from the injection site corresponding to their prolong T_{max} of 8-15 h. The slow absorption rate of EPO after subcutaneous administration is consistent with the delayed absorption characteristics of a large glycoprotein in general. The absorption rate of Epokine® was slightly faster than those of Eprex® which also showed faster absorption rate than those of Recormon®. Their median T_{max} were 8 h (range, 8-12 h), 10 h (range, 8-15 h), and 12 h (range, 8-15 h) for Epokine® Eprex® and Recormon®, respectively. The T_{max} values of Eprex® and Recormon® obtained from this study were similar to T_{max} values reported in the product monograph (5-24 h and 12-28 h for Eprex® and Recormon®, respectively) (71, 72). However, these T_{max} values were slightly faster than those values for epoetin alpha (8-24 h) and epoetin beta (8-36 h), reported previously (47, 70). The reason may be due to the change in other ingredients of drug formulation such as removal of the human serum albumin (HSA) stabilizer from epoetin alpha, in order to comply with new regulation from the European regulatory authorities for their concern of Mad Cow Disease (73). Polysorbate 80 and glycine were added instead of HSA for epoetin alpha. The formation of epoetin beta is HAS-free, but the stabilizer composition differs from that of epoetin alpha. Nonetheless, the result complies with previous finding in a delayed absorption rate of epoetin beta when compared to epoetin alpha (70).

The average $t_{1/2}$ of Epokine® and Eprex® were similar (34.9 h range 24-58 h vs 33.3 h range, 22-44 h, $p>0.5$) and when compared to those of Recormon® (38 h range, 20-49 h), no difference was found between the $t_{1/2}$ of Epokine® and Recormon® ($p>0.05$), however, the $t_{1/2}$ between Eprex® was statistically faster than those of Recormon® ($p<0.05$). In fact the half-life is difficult to evaluate after subcutaneous route due to the complexity and differences in drug absorption and elimination profiles. Moreover, the average $t_{1/2}$ of the three EPO preparations obtained from our study were longer than those values previously reported. Nonetheless, the result comply with the characteristic $t_{1/2}$ after subcutaneous dose of epoetin alpha (19.4 h range 6.9-48.3 h) which was faster than those of epoetin beta (24.2 h range 8.4-50.7 h) (70). The average mean residence time (MRT) for Epokine®, Eprex® and Recormon® by subcutaneous route were 57 h, 51 h and 61 h, respectively. Compared to previous

study, the MRT after subcutaneous route were 6-7 times longer than the average MRT after i.v. administration (6.8 h and 8.8 h for epoetin alpha and epoetin beta, respectively) (70). The total clearance (CL/F) of Epokine®, Eprex® and Recormon® were 19.4 mL/h/kg, 23.1 mL/h/kg, and 26.8 mL/h/kg, respectively. The clearance of EPO after subcutaneous route was very slow when compared to i.v. route (7.9 mL/h/kg and 8.1 mL/h/kg for epoetin alpha and epoetin beta, respectively). Systemic clearance of EPO was very low and the average value was approximately only 2-3 % of the liver blood flow (approximately 1,200 mL/h/kg) and 30% of the GFR. Similar to the $t_{1/2}$, the MRT and the CL/F between Epokine® and Eprex® were not significantly different ($p>0.05$), however, these pharmacokinetic parameters of Eprex® was statistically different from those of Recormon® ($p<0.05$). We therefore proposed that the elimination profiles of Epokine® fell between those of Eprex® (the fastest) and Recormon® (the slowest).

The mean volume of distribution (Vd/F) for Epokine® (56 L) was similar to Eprex® (64 L) ($p=0.7$) and both products had smaller Vd/F than those of Recormon® (85 L) ($p<0.05$). The average Vd/F for epoetin alpha and bata from our study were comparable to the previous study (63 L and 70 L respectively) (70). The Vd of EPO was slightly more than volume of the total body water (42 L) and the levels of EPO have been found to be almost 1.7 times higher in bone marrow than in plasma after a single i.v. dose of radio-iodinate recombinant EPO to rats (74). The larger Vd and a longer elimination $t_{1/2}$ of EPO beta compared with EPO alpha may be related to differences in their carbohydrate pattern (70). Halstenson et al. also hypothesize that EPO beta possess a slightly greater and a longer reticulocyte response than that of EPO alpha and that its larger Vd and longer terminal $t_{1/2}$ may be related to preferential binding to bone marrow (70). However, it can be argued that the larger Vd of beta EPO may account in part form its longer elimination $t_{1/2}$ and a more prolonged absorption phase than its alpha form. Furthermore similar to other biological products, following Sc injection, parts of EPOs may form complex and precipitate or deposit at the injection site leading to a lower bioavailability and a sustained absorption. The benefit is that subcutaneous EPOs resulted in a greater efficacy and is feasible for twice weekly injection recommended by the manufacturers which is more economic to and encourages greater compliance from the patients.

From the plasma concentration-time profiles, the EPO levels increased slowly after subcutaneous administration of the three preparations, attained peak concentrations at 8-15 h and were decreased gradually. The levels of EPO showed a plateau pattern after the peak and the level remain twice higher than the base-line EPO levels until 72 h after administrations. Although there are inter-subject variability of the concentration-time profiles, the pairwise intra-individual concentration-time profiles of Epokine®, Eprex® and Recormon® were almost identical for volunteer No. 4, 11, and 12. Volunteer No. 2, 9 and 10 showed higher profiles of Epokine® but the profiles for Eprex® and Recormon® were similar. Volunteers No. 3, 7 and 8 showed similar profiles between Epokine® and Eprex® which were higher than those of Recormon® and only volunteer No. 1, 5 and 6 showed the concentration-time profiles which were similar to the mean value.

The average C_{max} for Epokine® was the highest while the second was those of Eprex® and the lowest value was for Recormon®. The 90% CI for the ratios of C_{max}, between Epokine®/Eprex®, Epokine®/Recormon® and Eprex®/Recormon® were 1.10-1.50, 1.37-1.96 and 1.12-1.46, respectively. The 90% CI of the C_{max} between the three preparations were outside the 0.80-1.25 limits accepted for bioequivalence, but close to it. Since the absorption of epoetin after subcutaneous route is delayed and variable, the C_{max} which is considered either as a magnitude or as a rate of absorption may not accurately described either process. Other parameters such as MRT which is a measure of the average time the number of molecule introduced reside in the body and after extravascular dose is the sum of the MRT in the body and the mean absorption time may be use to describe the absorption rate of the drug.

Regarding the AUC_{0-t}, the 90%CI for Epokine®/Eprex®, Epokine®/Recormon® and Eprex®/Recormon® were 1.13-1.24, 1.29-1.45, 1.08-1.23, respectively. Based on the standard bioequivalence criteria, the AUC_{0-t} of Epokine® was higher than those of Recormon® while bioequivalence with Eprex®, and the AUC of Eprex® was bioequivalence with Recormon®. Our result was different from another study in dogs which found that the total AUC of Epokine®, Eprex® and Recormon® are not significantly different (75).

Our study demonstrated that the pharmacokinetic parameters of EPO are different among the beta form, the alpha form and the biosimilar product. Several

factors may attribute to these differences such as the fact that EPO exists as mixtures of isoforms which differed in their glycosylations (76), variations with their source, different recombinant DNA-derived human EPOs (38, 76), the type of cell in which the EPO is synthesized, the culture conditions used (77, 78), and the isolation procedures used for purification of the EPO as in the case of glycoprotein luteinizing hormone (79). In earlier studies, EPO preparations from different manufacturers have been shown to differ in their isoform composition, as judged by isoelectric focusing, electrophoresis, and lectin-binding assays (34, 38, 76). However, the above mentioned characteristic properties of the three EPO preparations were not well elucidated and it can be assumed that these differences are also existed with these preparations. To date, there are no valid reports documenting any significant differences between Eprex® and Recormon® with regards to their biological actions and clinical responses (54, 80), therefore the differences in pharmacokinetics may not fully explained the dynamic of the drug. We therefore propose that Epokine® is a candidate for further clinical study in patients to confirm its efficacy and safety.

CONCLUSION

In conclusion, the present study demonstrated the pharmacokinetic profiles of the three EPO preparations after subcutaneously administration a single dose of 4,000 IU in healthy Thai male volunteers. The two alpha forms are more closely related in terms of their pharmacokinetics than the beta form. Although there are no valid reports documenting any significant differences between Eprex® and Recormon® with regards to their clinical efficacy to raise reticulocytes in renal anemia, it remains to be seen whether Epokine® will demonstrate the same clinical response to the currently available EPO preparations in spite of their different pharmacokinetic profiles.