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Rapid communication

An in vitro study of epoetin β intravenous injection site at the end of hemodialysis

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ABSTRACT

The aim of this study (SITEPO™) is to evaluate the influence of the intravenous injection site (drip chamber injection site, venous injection site or venous fistula needle) on plasma concentration of epoetin β (Neorecormon®, Roche), a recombinant Human Erythropoietin (rHuEPO), at the end of *in vitro* hemodialysis sessions. No practical administration guidelines are available.

Twenty 1-h dialysis sessions are performed. Before each dialysis, the circuit is filled with 270 ml, of heparinized total human blood whose hematocrit is adjusted to 35%. A common dosage of epoetin β in clinical practice (3000 IU) is studied for the three injection sites and for reference experiments in which rHuEPO is not injected into the dialysis circuit. Plasma concentrations of erythropoietin are measured by ELISA. The physiologically endogenous erythropoietin concentration is systematically determined and removed from the total epoetin β concentration.

Average epoetin β plasma levels returned are not significantly different between the three injection sites and no significant rHuEPO loss is observed after injection into the drip chamber, the venous injection site and the venous fistula needle compared with reference experiments.

The three intravenous injection sites of rHuEPO can be used at the end of dialysis without significant epoetin β loss.

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Recombinant Human Erythropoietin (rHuEPO) is an effective treatment for anaemia in patients with chronic renal failure in predialysis and those undergoing hemodialysis or peritoneal dialysis (Dunn and Markham, 1996; Macdougall, 2003). In hemodialysis patients, rHuEPO is often injected intravenously (IV) as a bolus injection during or at the end of hemodialysis sessions because of ease of venous access and for patient comfort (Patel et al., 2007).

One of the few studies available, an *in vivo* study (Petersen et al., 1996), compared the plasma levels of rHuEPO after injection into the venous drip chamber at the beginning of the dialysis to the level after injection into the venous injection port. This study showed that the injection site can affect the plasma level. Substantial quantities of rHuEPO were lost when injected into the Fresenius A2008 venous drip chamber because trapping occurred and could not be eluted at the end of dialysis. No significant loss of EPO was observed with the cartridge drip chamber of the Cobe Centry 3 delivery system. Venous drip chambers have been modified since this study, and no other study has yet been conducted on this subject.

The objective of our SITEPO™ study is to determine, after 1-h simulated dialysis sessions, if the epoetin β (Neorecormon®) injection site influences the plasma level of EPO returned at the end of dialysis. The three rHuEPO injection sites commonly used are the venous drip chamber injection site, the venous injection site, or the venous fistula needle.

The SITEPO™ study includes twenty experiments consisting of twenty 1-h simulated dialysis sessions. Neorecormon® 3000 IU/0.3 ml pre-filled syringes (Roche) is the chosen dosage for this study. The experiment is repeated five times for the three injection sites. A reference study has been conducted with five experiments where EPO β is not injected into the dialysis circuit but directly into the blood collected after restitution.

Each dialysis session is performed using the AK200S® delivery system (Gambro) with a FX8® (Fresenius) polysulfone membrane as well as a A100DG® (Gambro) arterial line, a Bioline GH-V10G® (Hospal) venous line, and a PLUME S® (Hospal) venous fistula nee-

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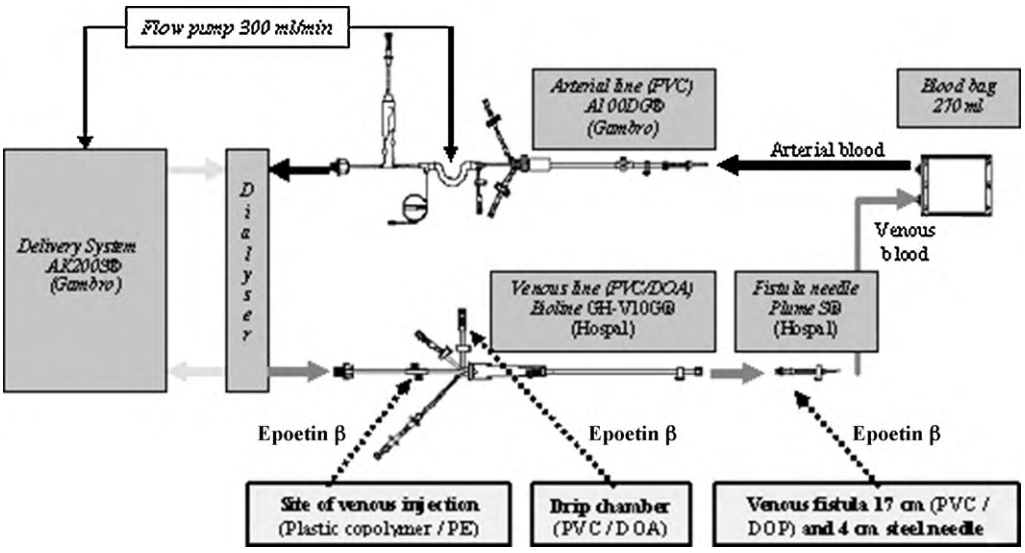

Fig. 1. Hemodialysis circuit in dialysis mode with a 300 ml/min flow and epoetin β injection sites (PVC: polyvinyl chloride, DOA: dioctyladipate, DOP: dioctylphtalate, PE: polyethylene).

dle. The venous line is composed of a drip chamber and an injection site located between the dialyser and the drip chamber. The venous fistula needle is located at the end of the venous line (Fig. 1).

The dialysis solution is produced by the generator supplied with osmosed water, with acid concentrate Clear Flex 7076® (Baxter) and a bicarbonate cartridge Bicart® (Gambro). The circuit is rinsed with 2 l of pre-heparinized 0.9% sodium chloride Prontoprime® (Baxter) before being filled with total blood. Flow rate is 100 ml/min. Arterial and venous pressure sensors are stabilized with an opposing air pressure.

Total human blood from anonymous healthy volunteers and rejected for inadequate volume (French blood establishment of Aquitaine-Limousin) is used. For each dialysis, a bag containing 270 ml of fresh total blood from different patients is prepared: hematocrit is adjusted beforehand to 35% (for total blood donations whose hematocrit is above 35%) with 0.9% sodium chloride (Analyzer ELECTROLYTE 8® Nova Biomedical) and 1000 IU heparin are added. The blood bag is connected to the arterious line and the circuit filled with a flow of 100 ml/min. When filling is completed, the blood bag is connected to the venous line by the fistula needle (rubber connection directly punctured). The dialysis session can begin in a closed circuit during 1 h (blood flow 300 ml/min, null ultra-filtration rate).

EPO β is injected into either the venous drip chamber (without using the needle of the pre-filled syringe) or into the venous injection site (using the syringe needle) at the end of dialysis just before

Table 1
EPO β injected into the venous injection site, the venous drip chamber, the venous fistula needle and not injected into the dialysis circuit: total quantity of EPO β (IU) returned in the plasma at the end of each dialysis.

Injection site of EPO β (3000 IU)	Dialysis order	Blood Hct[a] after return (%)	Total quantity of returned blood after dialysis (ml)	Total quantity of endogenous EPO returned in plasma (IU[b])	Total quantity of EPO β returned in plasma (IU[b])	Average total quantity of EPO β returned in plasma (IU[b] ± SD[c])
Venous injection site	2	18	249.06	2.72	2540.13	2902.14 ± 301.12
	6	29	249.45	0.49	3054.28	
	9	31	248.87	2.26	2662.62	
	13	32	248.68	1.02	3285.21	
	18	30	248.78	1.15	2968.43	
Venous drip chamber	1	28	246.67	1.49	2420.27	2618.96 ± 210.71
	4	22	249.26	1.14	2481.48	
	8	29	248.59	0.91	2646.59	
	12	31	248.68	1.50	2584.96	
	16	26	248.59	1.44	2961.47	
Venous fistula needle	3	28	249.16	0.52	2346.91	2825.76 ± 271.55
	7	28	249.64	0.66	2942.26	
	11	30	249.06	1.63	3008.72	
	14	28	248.97	0.98	2879.77	
	19	31	249.06	0.88	2951.12	
Reference experiments	5	20	248.59	0.57	2778.06	2892.10 ± 262.58
	10	25	248.59	1.24	2617.21	
	15	27	248.87	0.58	3233.69	
	17	26	248.78	0.91	3102.10	
	20	32	248.49	1.23	2729.43	

[a] Hematocrit.
[b] International unit.
[c] Standard deviation.

blood restitution or directly into the venous fistula needle plunging into a glass collecting erlenmeyer, after blood return, without using the syringe needle. Each EPO β injection is rinsed by a consecutive injection of 10 ml 0.9% sodium chloride. For the five reference experiments, EPO β is not injected into the dialysis circuit but directly into the blood collected after restitution. For each experiment, before each EPO β injection, a 6 ml blood sample is collected in order to determine plasma endogenous EPO concentrations.

The blood restitution at the end of dialysis was carried out with sodium chloride 0.9% with a flow rate of 100 ml/min in the erlenmeyer. A standardized restitution of precisely 260 g of blood (250 ml), essential to the reproducibility of the experiments, is made with blood magnetic shake. After each EPO β injection and blood restitution, a new 6 ml blood sample is collected in order to determine plasma EPO β concentration and final blood hematocrit.

A glass collecting erlenmeyer for final blood restitution would appear to be more advantageous for its neutrality and to avoid a possible sorption of the drug in the collecting container as observed in our preliminary experiments (data not shown). Some drugs, such as anti-depressive agents, anaesthetics, benzodiazepines, anticoagulants and anti-angorous agents showed adsorption on PVC bags and an absence of adsorption on glass containers (Martens et al., 1990; Airaudo et al., 1998; Sattler et al., 1998).

Blood samples collected are centrifuged and plasma samples stored at −20 °C. Plasma erythropoietin concentrations expressed in International Units (IU)/ml of plasma are determined by a validated Enzyme Linked Immunosorbent Assay (ELISA Kit Quantikine® IVD® R&D Systems Europe).

Results are given as the mean ± standard deviation (SD) total quantity of EPO β returned determined after injection into the venous injection site, the drip chamber, the venous fistula needle or during the reference experiments (Table 1). These values are calculated with total plasma quantities of EPO β returned at the end of each of the twenty dialysis sessions and expressed in International Units (IU). The total plasma quantity of EPO β is obtained for each dialysis from the plasma concentration of total EPO (i.e. EPO β plus endogenous EPO) minus the corresponding plasma concentration of endogenous EPO before EPO β injection and reported to the total volume of returned plasma. The total volume of returned plasma corresponds to the total volume of returned blood, minus red blood cell volume determined with blood hematocrit measured after blood restitution. Variance analysis on individual data is used for the comparison of the four means and two Student's *t*-tests are used to confirm the results.

The comparison of the four calculated average total quantities of EPO β (Table 1) shows no significant difference according to the injection site (variance analysis on individual data).

However, the average return level of EPO β tends to be lower after injection into the drip chamber. In fact, the mean loss of EPO β returned after drip chamber injection compared with the reference experiment is 273.14 IU (9.4% loss). The mean loss of EPO β returned after venous fistula needle injection and after venous injection site compared with the reference experiment are 66.34 IU (2.3% loss) and null respectively. The absence of significant difference between drip chamber and others injection sites is confirmed with two Student's *t*-tests: the mean total quantity of EPO β returned after injections into the drip chamber (mean of five experiments) is firstly compared to the mean total quantity of EPO β returned after all the other fifteen experiments ($p < 0.1$), and secondly to the mean total quantity of EPO β returned with the five reference experiments ($p < 0.2$).

The interest of an *in vitro* study is the reproducibility of the results (dialysis conditions and injection technique standardized) and the reliability to identify rHuEPO losses with reference experiments.

Regarding the non-significant EPO β loss (9.4%) when injected into the drip chamber, pure EPO adsorption by direct contact with the PVC tube (PVC containing 28% dioctyladipate as plasticizer) may not be an important factor in EPO β loss: indeed, the loss when directly injected into the fistula needle (PVC containing 45% dioctylphtalate as plasticizer) is only 2.3% compared with reference experiments. Moreover, the drip chamber and the fistula needle only differ by their plasticizer (dioctyladipate and dioctylphtalate) and two studies with isosorbide mononitrate (De Muynck et al., 1990) and dinitrate (De Muynck et al., 1991) have shown that the type of PVC plasticizer may not influence the adsorption of drugs. This non-significant difference observed is probably due to the experimentations and significant EPO β adsorption on these materials can be excluded. EPO β adsorption is also excluded on polyethylene and plastic copolymer constituting our venous injection site.

With the comparison of reference experiments, where EPO β has not been in contact with the dialysis circuit, and experiments in which EPO β is injected into the dialysis circuit, we observe and conclude that in patients with chronic dialysis, the three injection sites of a common use venous line, composed of polyethylene, plastic copolymer and polyvinyl chloride with dioctyladipate or dioctylphtalate as plasticizers, can be used without significant loss for intravenous injection of epoetin β at the end of dialysis. The results of this study need to be now confirmed *in vivo*.

Conflicts of interest

None.

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