Hyperhydration effect on pharmacokinetic parameters of recombinant human erythropoietin in urine and serum doping control analysis

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Introduction

Despite their use as therapeutic agents, rHuEPO and analogues are known to be abused by athletes with the aim of improving endurance performance due to the increase of hemoglobin and subsequently blood oxygen carrying capacity [1]. Recently, it has been noticed that some athletes consume unusual high volumes of water or sports drinks before anti-doping sample collection and it is possible that the aim of this practice is to manipulate plasma volumes or urinary excretion, since dilution based on drinking is not prohibited as a practice according to WADA`s current regulations [1].

Objectives

To conduct an exploratory trial on limited number of healthy volunteers based on preliminary simulation study [2] in order to:

- ✓ Investigate the influence of hyperhydration on the blood (serum) and urinary PK profiles of rHuEPO;
- ✓ Perform compartmental PK modeling and NCA PK analysis to describe serum and urinary rHuEPO concentrations after a single dose administration of Epoetin beta;
- ✓ Examine the reliability and robustness of the routine anti-doping SAR-PAGE analysis on the detection sensitivity of rHuEPO under hyperhydration conditions.

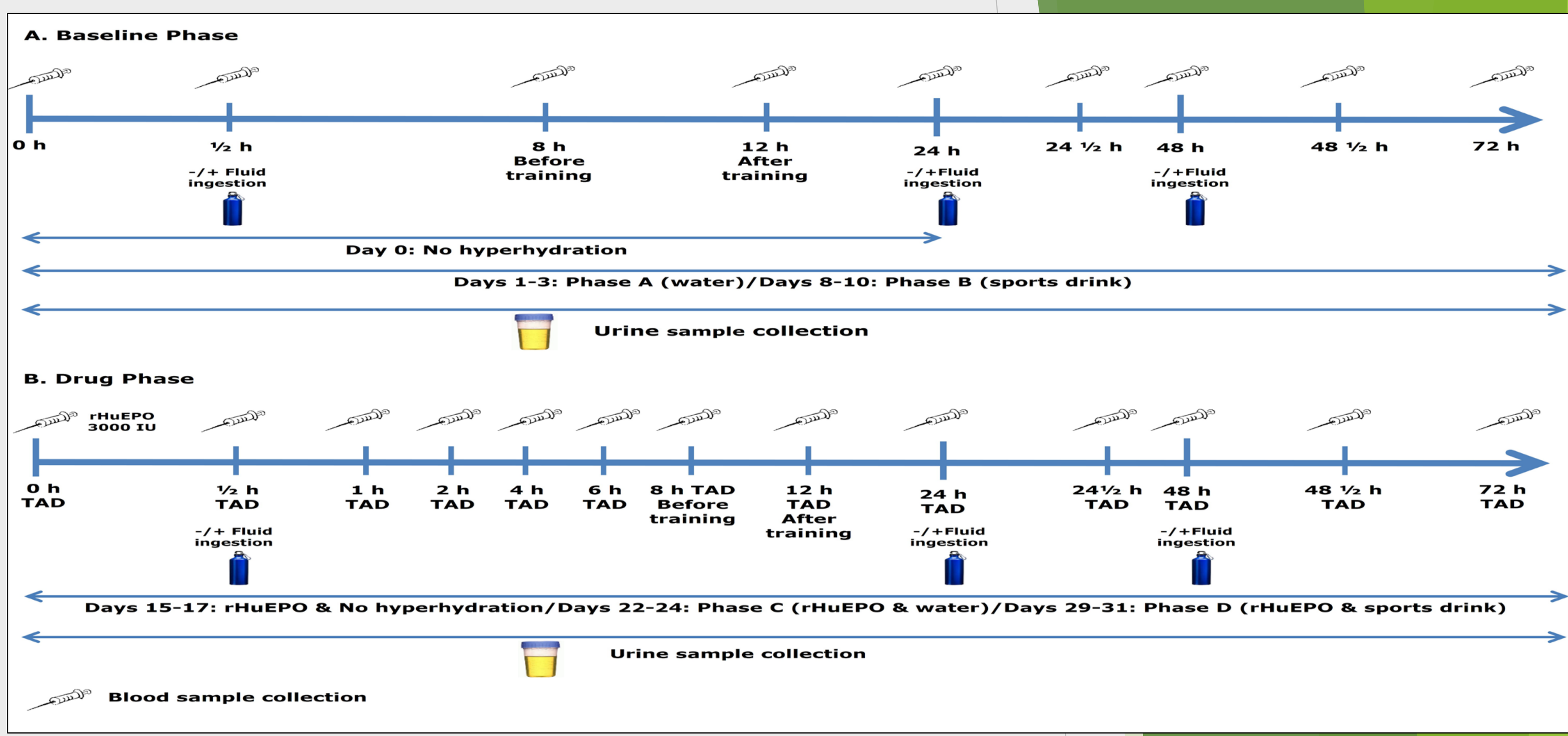
Methodology

Population: Seven healthy, recreationally active non-smokers Caucasian male volunteers (38.4 ± 8.14 years, 27.6 ± 3.62 kg/m^2 BMI and 136 ± 19.0 mL/min CrCL). Written consent was provided by all the subjects prior to participation and were free to withdraw from the study at any time. The study was approved by the Ethics Committee of the Anti-Doping Lab Qatar, Doha, Qatar.

PK analysis: Population PK modeling was performed on the measured serum concentrations after rHuEPO administration using Monolix® software version 2018a (Lixoft, Batiment D, Antony, France), while non-compartmental analysis (NCA) PK analysis was performed on the measured serum and urinary EPO concentrations using the Phoenix® version 8.0 PK/PD software package (Certara, Princeton, NJ, USA). One and two compartment models together with various absorption models which included zero and first order, separately and combined, with and without lag time, as well as different error models were tested. Inter-individual variability (IIV) was considered to be univariate log-normal for all the parameters. Age and BW were tested as covariates on the parameters of the final model.

Statistical analysis: Data distribution was tested using the Shapiro-Wilk (S-W) normality test. Significance was set at $P < 0.05$ level and all tests were two-tailed with 95% CI. Statistical data analysis was performed by SPSS version 25.0 (IBM SPSS Statistics for Windows, Version 25.0, IBM Corporation, Armonk, NY, USA) software package.

Figure 1: Experimental study protocol and sample collection.

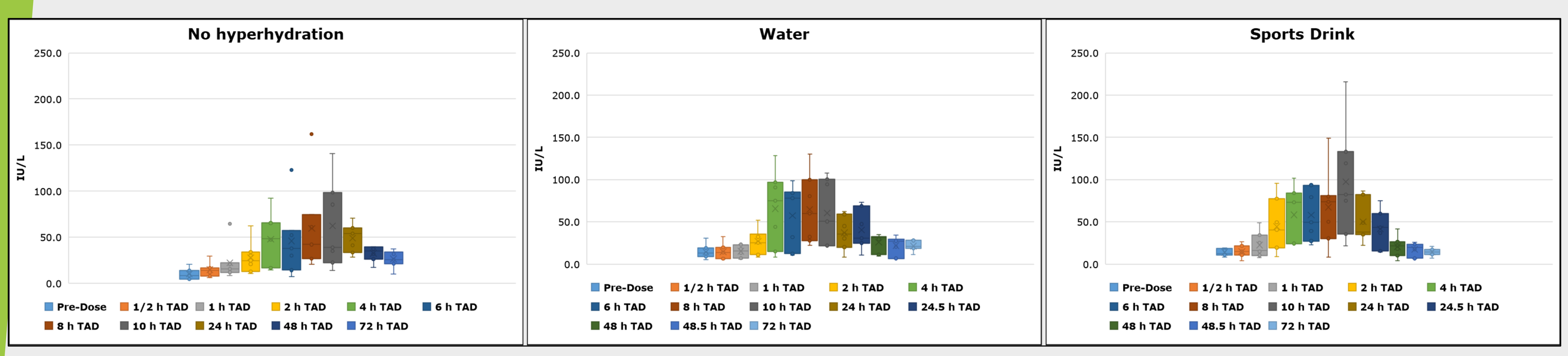


Results and Discussion

Serum and urinary EPO concentrations

- ✓ Baseline serum EPO concentrations ranged from 7.81 to 34.1 IU/L (median value 15.2 IU/L), while urinary endogenous EPO concentration levels were within 0.79–3.17 IU/L (median 1.32 IU/L) following the rhythm of urinary production;
- ✓ After SC rHuEPO administration, measured serum EPO concentrations did not present any dilution effect (**Fig. 2**);
- ✓ Serum and urinary EPO concentration-time profiles (**Fig. 2**) after SC rHuEPO administration and NCA calculated PK parameters (**Table 1**) remained unaffected (*$P > 0.05$*) after excessive fluid intake (water or sports drink administration);
- ✓ Urinary EPO concentration ranged between 0.69 and 4.78 IU/L (population median 1.20 IU/L) reaching C_{max} (2.52 ± 1.04 IU/L) after 17.5 ± 11.3 hours. Median values of 1.53 IU/L (range 0.79–3.88 IU/L) and 1.61 IU/L (range 0.77–5.58 IU/L) were found for the water and sports drink, respectively (**Table 2**);
- ✓ RCL of endogenous and rHuEPO was increased approx. twice after hyperhydration supporting the non-significant difference on the urinary concentrations (**Table 2**).

Figure 2: Box plots of Epoetin beta (IU/L) in serum samples up to 72 hours after subcutaneous administration.



Serum and urinary PK parameters

- ✓ One compartment PK model with zero order absorption adequately described EPO kinetics in all the phases (under normal & hyperhydration conditions), parameterized as total clearance (CL), volume of distribution (V_d) and time for zero order absorption (Tk_0);
- ✓ An "Endogenous EPO" parameter was introduced to the base PK model to account for the endogenous EPO concentration measured with the exogenously administered rHuEPO and a lag time (T_{lag}) to describe the delayed absorption observed after hyperhydration;
- ✓ A multiplicative residual error was used in all cases;
- ✓ No covariates were found to improve the base model probably due to the small number of individuals enrolled in the study;
- ✓ Despite a slight C_{max} overestimation for Phase C, a good model prediction was observed for all the phases of rHuEPO administration (normal hydration conditions, Phases C and D) with acceptable %RSE and IIV% (RSE%) for PK parameter estimates and residual error < 30% in all cases (**Table 3, Fig.3**).
- ✓ Apart from the observed delayed absorption after water and sports drink hyperhydration, a trend for decreased V_d and increased CL values was also observed. In addition, higher endogenous EPO was found after extensive fluid ingestion (**Table 3**).

Table 3. PK parameter estimates and their IIV estimates, with standard error (SE) expressed as percentage relative standard error (RSE%).

Study Phase	PK Parameter	Estimate (RSE%)	IIV% (RSE%)
rHuEPO	Endogenous EPO (IU/L)	8.51 (16.8)	37.3 (35.4)
	Tk_0 (h)	6.96 (31.2)	74.5 (35.4)
	V_d (L)	63.1 (27.2)	67.1 (29.9)
	CL (L/h)	0.775 (14.6)	14.5 (73.3)
	Residual error (%)	27.0 (10.1)	-
rHuEPO & Water	Endogenous EPO (IU/L)	13.1 (19.9)	48.1 (30.1)
	T_{lag} (h)	1.39 (33.3)	59.3 (46.5)
	Tk_0 (h)	4.10 (35.5)	67.2 (41.4)
	V_d (L)	53.4 (29.1)	66.2 (34.7)
	CL (L/h)	2.22 (40.8)	98.2 (33.5)
	Residual error (%)	26.4 (9.82)	-
rHuEPO & Sports Drink	Endogenous EPO (IU/L)	12.6 (14.2)	32.8 (31.9)
	T_{lag} (h)	0.66 (31.3)	39.1 (76.3)
	Tk_0 (h)	11.6 (23.9)	34.7 (55.7)
	V_d (L)	24.7 (37.2)	80.3 (33.3)
	CL (L/h)	2.03 (25.5)	63.1 (29.9)
	Residual error (%)	28.1 (9.43)	-

Figure 3: Measured (DV) versus predicted (IPRED) serum erythropoietin (EPO) concentrations (upper panel) and Visual Predictive Check (VPC) plots (lower panel) after recombinant human erythropoietin (rHuEPO) administration per study phase (left panel: no hyperhydration, middle panel: Phase C: rHuEPO & water, right panel: Phase D: rHuEPO & sports drink).

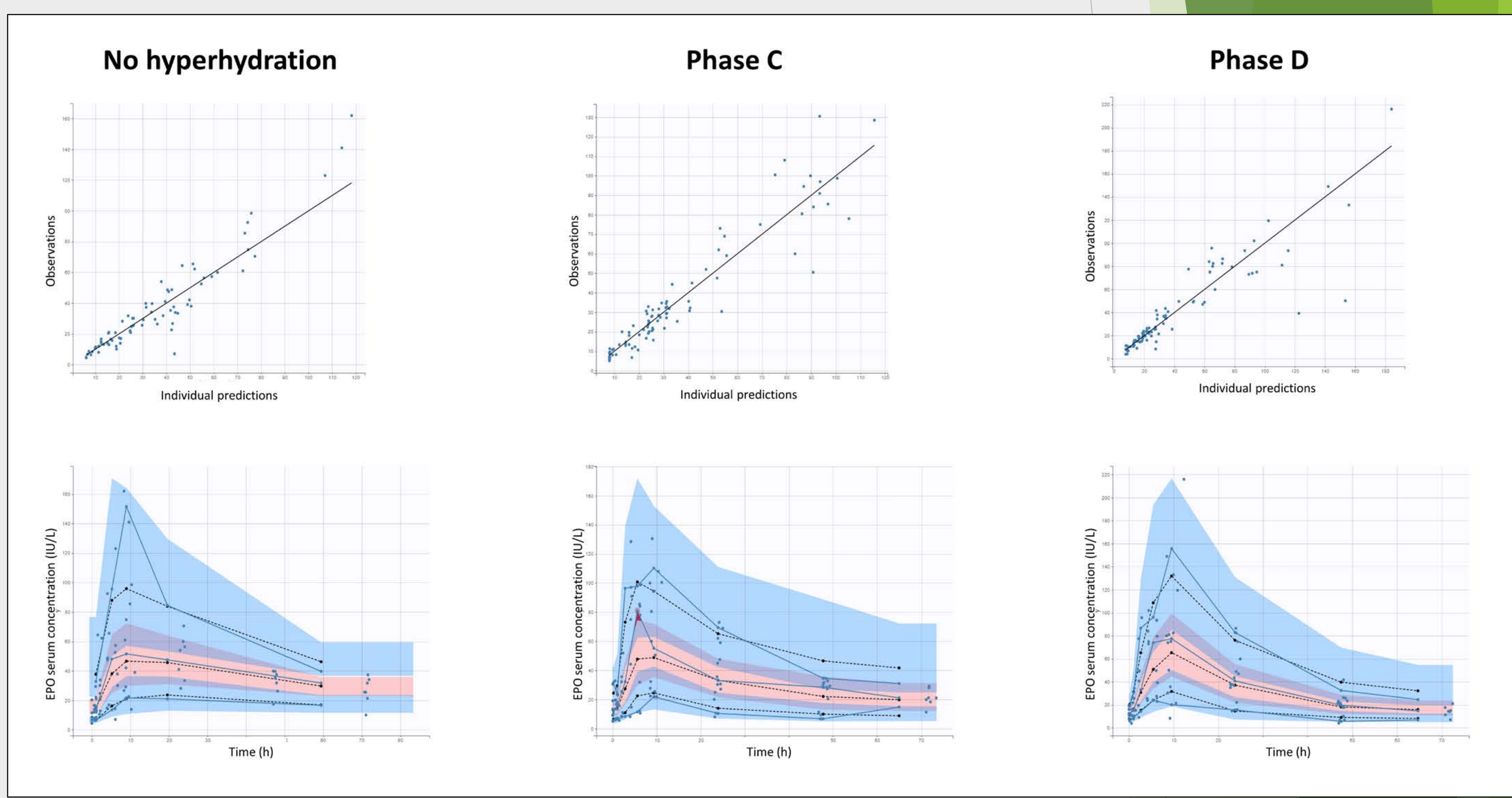


Table 1. Non-compartmental serum PK parameters expressed as mean ± SD.

Study Phase	C_{max} (IU/L)	AUC_{0-24h} (h*IU/L)	AUC_{0-72h} (h*IU/L)	AUC_{inf} (h*IU/L)	T_{max} (h)	$t_{1/2}$ (h)	MRT (h)
rHuEPO	76.4 ± 44.9	1203 ± 671	2865 ± 994	4729 ± 1877	14.5 ± 8.02	46.8 ± 19.2	31.5 ± 3.84
rHuEPO & Water	77.3 ± 42.2	1173 ± 650	2490 ± 1130	3780 ± 1038	10.7 ± 6.12	44.9 ± 22.8	29.7 ± 4.37
rHuEPO & Sports Drink	104 ± 63.5	1545 ± 830	2671 ± 1208	3345 ± 1177	11.5 ± 5.63	31.1 ± 10.9	24.8 ± 4.59

Table 2. Urinary C_{max}, T_{max}, cumulative amount ($ACum_{0-t}$) and renal clearance (RCL_{0-t}) values with and without rHuEPO administration under normal hydration and after excessive fluid ingestion expressed as mean ± SD.

Study Phase	C_{max} (IU/L)	T_{max} (h)	$ACum_{0-24h}$ (IU)	$ACum_{0-72h}$ (IU)	RCL_{0-24h} (L/h)	RCL_{0-72h} (L/h)
Baseline	2.26 ± 0.588	7.91 ± 5.88	3.31 ± 0.984	9.93 ± 2.95	0.003 ± 0.001	0.003 ± 0.001
Water	1.82 ± 0.147	16.7 ± 10.9	4.91 ± 1.20	15.0 ± 2.11	0.013 ± 0.005	0.013 ± 0.005
Sports Drink	1.85 ± 0.193	40.2 ± 20.2	4.59 ± 1.41	14.1 ± 3.27	0.011 ± 0.005	0.012 ± 0.004
rHuEPO	2.52 ± 1.04	17.5 ± 11.3	2.98 ± 1.00	8.16 ± 2.70	0.003 ± 0.002	0.003 ± 0.002
rHuEPO & Water	2.44 ± 0.759	10.6 ± 9.26	5.71 ± 0.898	15.3 ± 2.34	0.007 ± 0.004	0.008 ± 0.006
rHuEPO & Sports Drink	3.16 ± 1.30	19.7 ± 15.6	6.00 ± 1.06	15.4 ± 2.10	0.005 ± 0.002	0.007 ± 0.004

Conclusion

- ✓ Serum and urinary EPO concentration-time profiles remained unaffected after excessive fluid intake.
- ✓ Serum and urinary PK parameters of a WADA prohibited substance were utilized for the first time in conjunction with the urinary concentration-time profiles as a tool for doping detection.
- ✓ The reliability and robustness of the routine anti-doping analysis of rHuEPO was verified under the applied study protocol.

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