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Targeted temperature management after cardiac arrest is associated with reduced metabolism of pantoprazole – A probe drug of CYP2C19 metabolism

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ABSTRACT

Objective: Targeted temperature management (TTM) is part of standard post-resuscitation care. TTM may downregulate cytochrome enzyme activity and thus impact drug metabolism. This study compared the pharmacokinetics (PK) of pantoprazole, a probe drug of CYP2C19-dependent metabolism, at different stages of TTM following cardiac arrest.

Methods: This prospective controlled study was performed at the Medical University of Vienna and enrolled 16 patients following cardiac arrest. The patients completed up to three study periods (each lasting 24 h) in which plasma concentrations of pantoprazole were quantified: (P1) hypothermia (33 °C) after admission, (P2) normothermia after rewarming (36 °C, intensive care), and (P3) normothermia during recovery (normal ward, control group). PK was analysed using non-compartmental analysis and nonlinear mixed-effects modelling.

Results: 16 patients completed periods P1 and P2; ten completed P3. The median half-life of pantoprazole was 2.4 h (quartiles: 1.8–4.8 h) in P1, 2.8 h (2.1–6.8 h, $p = 0.046$ vs. P1, $p = 0.005$ vs. P3) in P2 and 1.2 h (0.9 – 2.3 h, $p = 0.007$ vs. P1) in P3. A two-compartment model described the PK data best. Typical values for clearance were estimated separately for each study period, indicating 40% and 29% reductions during P1 and P2, respectively, compared to P3. The central volume of distribution was estimated separately for P2, indicating a 64% increase compared to P1 and P3.

Conclusion: CYP2C19-dependent drug metabolism is downregulated during TTM following cardiac arrest. These results may influence drug choice and dosing of similarly metabolized drugs and may be helpful for designing studies in similar clinical situations.

1. Introduction

Targeted Temperature Management (TTM) at 32–36 °C is part of standard post-resuscitation care (after return of spontaneous circulation) as recommended by current guidelines [1]. TTM improves survival and neurological outcome in patients after successful cardiopulmonary resuscitation, [2,3] but also causes side-effects, such as a higher rate of respiratory infections [4]. Several studies indicate a substantially altered metabolism of certain drugs at lower body temperatures. For example, clearance of the CYP3A4-metabolized fentanyl was reduced by 46% in patients treated with TTM after cardiopulmonary resuscitation (CPR) when compared to case-matched critically ill patients [5]. The hypothesized mechanism is a reduced activity of enzymes belonging to the hepatic cytochrome system (CYP450) [4–9], which are critically involved in the metabolism of a multitude of drugs [5,6,10]. Aside from hypothermia, other factors including genetic polymorphisms, systemic

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inflammation, specific inhibitors or inducers also may affect enzyme activity and consequently lead to altered pharmacokinetics (PK) [11,12]. There are only limited data available on the PK of specific drugs during TTM after cardiac arrest. It is noteworthy that available studies included only small sample sizes and the chosen control groups (matched critically ill patients, patients not undergoing TTM or historic controls), were subject to obvious limitations making the interpretation of these studies difficult [5,6].

Pantoprazole is a proton pump inhibitor frequently used in intensive-care-unit (ICU) patients to reduce the risk of stress ulcers. It is metabolized via CYP2C19 enzymes [11]. In critically ill patients with a substantial systemic inflammatory response the terminal elimination half-life ($T_{1/2}$) of pantoprazole increased approximately five-fold [13]. Pantoprazole may be used as a "probe drug" for CYP2C19 dependent drug metabolism, which may be extrapolated to other substances mainly metabolized by this enzyme. Such drugs include clopidogrel, various antidepressants and anti-fungal drugs. [6,14].

The aim of this study was to compare the PK of pantoprazole in patients after successful CPR in three study periods: during TTM, after rewarming to normothermia in the ICU and, if possible, after the patient had regained consciousness and had (almost) made a full recovery on a normal ward. To overcome important limitations of prior studies, we evaluated pantoprazole PK in all three study periods in the same patient population.

2. Methods

2.1. Study design and setting

This was a prospective, longitudinal, monocentric trial to investigate the PK of pantoprazole in patients who were treated with TTM after achieving sustained return of spontaneous circulation after out-of-hospital cardiac arrest. The study was conducted at the Department of Emergency Medicine and the consecutively treating ICUs and normal care wards at the General Hospital of Vienna, Medical University of Vienna. Patients were screened on site and enrolled depending on inclusion and exclusion criteria and the availability of the study team. All enrolled patients were treated with TTM as a standard treatment and received pantoprazole treatment (40 mg intravenous bolus infusion per 24 h) as part of post resuscitation care, independently from the study. Prior to drug administration, a baseline blood sample was taken to identify a potentially unknown previous pantoprazole intake. Furthermore, all patients were genotyped for CYP2C19 polymorphisms and classified as intermediate (CYP2C19 *1/*2), extensive (CYP2C19 *1/*1), rapid (CYP2C19 *1/*17) and ultra-rapid metabolizers (CYP2C19 *17/*17) according to previous studies [11,13,15]. Baseline characteristics, including resuscitation-specific parameters, (e.g. age, initial rhythm, bystander resuscitation) were recorded in a way which has been previously published [16].

2.2. Study population

The study population consisted of patients who were admitted to the Emergency Department after out-of-hospital-cardiac arrest and met the following inclusion criteria: no contraindication for TTM, $\geq$ 18 years of age, treatment with pantoprazole. Exclusion criteria comprised increased inflammatory biomarkers (C-reactive protein (CRP) $>$ 5 mg/dL), life expectancy $<$ 3 days based on treating physicians' estimations, body temperature $<$ 34.0 °C at admission, known liver dysfunction (i.e. liver cirrhosis, history of significant liver disorders), chronic kidney failure, intake of known inducers or inhibitors of CYP2C19 or CYP3A4, allergies to, or intolerances against, pantoprazole, prior known pantoprazole treatment within 24 h and pregnancy or breastfeeding.

2.3. TTM after cardiac arrest

The current guidelines for post-resuscitation care recommend TTM with a body temperature of 32–36 °C for at least 24 h following cardiac arrest. [1] At our institution, all patients who are unconscious following cardiac arrest and do not demonstrate contraindications for TTM (e.g., uncontrolled bleeding or uncontrollable hemodynamic instability) are routinely treated with TTM at 33 °C. This is done in accordance with standard operating procedures: 24 h at 33 °C ± 1 °C. After 24 h, patients undergo a controlled rewarming period (0.5 °C /h) until normothermia is achieved (36.5 °C ± 1 °C). [17] Normothermia is maintained for at least 72 h by physical or pharmacological means. Within this study, all patients initially received infusion of cold fluids; twelve patients were subsequently cooled with Arctic Sun® 5000 Temperature Management System (Bard Medical Division, C.R. Bard, Inc. Louisville, CO, USA); two patients were treated via invasive cooling using the Thermogard XP® Temperature Management System (ZOLL Medical, Cologne, Germany) and two patients were cooled with surface cooling pads (EMCOOL-Spad®, Emcools AG, Pfaffstaetten, Austria) [18].

2.4. Study periods

Data collection and blood sampling were performed in three periods as follows:

The hypothermic period (period 1, P1): after reaching the target temperature (33 °C).

The normothermic period (period 2, P2): after reaching normothermia ((36.5 °C ± 1 °C).

The conscious period (period 3, P3): those patients who survived and regained consciousness (with favourable neurological outcome) and were able to give informed consent (n = 10) were asked to participate in a third study period in the absence of critical illness and pronounced systemic inflammation (CRP <5 mg/dL) or fever (=normothermia).

All patients received an intravenous bolus of 40 mg pantoprazole. (HIKMA FARMACEUTICA S.A.).

2.5. Blood sampling and processing

During all three periods, blood samples were obtained at baseline (=before drug dosing) and at 15 min, 1 h, 2 h, 4 h, 8 h and 24 h after pantoprazole infusion. Pantoprazole was infused as soon as the respective body temperature was reached in each period. Immediately after the blood samples (3 mL spray-coated lithium heparin tubes) were drawn, they were cooled to 4 °C, centrifuged (10 min at 2000 G) and the plasma was stored at − 80 °C until batch analysis was performed. The plasma concentration of pantoprazole was quantified using liquid chromatography tandem mass spectrometry. The method was developed based on the method published by Li, Y. et al. [19] and is described in detail in the Supplements.

2.6. Non-compartmental analysis

The primary endpoint of this trial was the $T_{1/2,}$ in each study period. Secondary endpoints included the area under the time-concentration curve (from baseline (=pre-dose) until the last measured value at 24 h, $AUC_{0\text{-last}}$), the volume of distribution (Vd, total Vd and normalized per kg bodyweight) and clearance (CL, total CL and normalized per kg bodyweight) of pantoprazole. Non-compartmental PK analysis was calculated using Phoenix WinNonlin (Certara, New Jersey, USA). For a detailed explanation on parameter calculation please refer to the Supplement.

2.7. Statistical analysis and sample size calculation of the non-compartmental analysis

Although the PK of pantoprazole is well-known in normothermic

patients, the effect of TTM on CYP2C19-dependent drug metabolism is currently unknown. Assuming a terminal elimination half-life of "normal" metabolizers of 1.3 ± 0.4 h and a 50% increase, a sample size of 7 patients per group would suffice to show a significant difference with a power of 80% and an alpha of 5% [11]. To increase the power to 90%, a sample size of 10 per group should suffice. Based on data from critically ill patients showing marked differences in the PK of pantoprazole, and to account for potential dropouts (patients not completing all study periods), we chose a sample size of 16 [13]. An exact prediction of the number of patients who would complete all study periods was not possible beforehand due to unclear neurologic outcome of individual patients, and the requirement of obtaining informed consent before P3. PK parameters are presented using descriptive statistics (medians and quartiles).

The primary endpoint ($T_{1/2}$) was compared between study periods by conducting a Friedman ANOVA including all study periods. In a second step, study periods were compared pairwise by a Wilcoxon-test for exploratory analyses. By implementing a hierarchical testing strategy, corrections for p-values were not performed. Secondary endpoints included the AUC, Vd and CL. These endpoints were compared by a Friedman ANOVA and Wilcoxon-test in an exploratory manner. For the overall comparison of the three periods, and for the pairwise comparison with P3, only 10 patients were available and included in the analysis. A comparison of P1 and P2 with all 16 patients is presented in the supplement. A two-sided alpha of < 0.05 was used as significance level for the primary endpoint analysis.

Statistical analysis was performed with IBM SPSS Statistics (version 26, IBM, Endicott, NY, USA). Figures were drawn using Graphpad Prism (version 8.4.3, San Diego, CA, USA).

2.8. Population PK model development

Nonlinear mixed-effects modelling were used to describe pantoprazole PK and to quantify the variability in PK between the study periods. One- and two-compartment models were evaluated. Inter-individual variability (IIV) was tested on all structural model parameters. To estimate the within-subject variability in PK parameters across the three study periods, we separated the PK samples into three groups based on the study period in which they were drawn. We then assessed whether inter-occasion variability (IOV) could be quantified separately for each of the PK parameters. Both IIV and IOV were assumed to be log-normally distributed. Additive, proportional and combined error models were evaluated for the residual variability. Due to the small number of patients in this study we used a priori allometric scaling of all PK parameters based on body weight, rather than testing covariate relationships based on body size empirically [20–22]. Based on the hypothesized mechanisms that may influence pantoprazole PK, we tested body temperature as covariate on clearance (CL) and CRP levels as covariate on CL and the central volume of distribution (Vc) using linear and power functions. In a parallel covariate analysis, we tested the potential effect of the three study periods on CL and Vc by estimating a factor by which the PK parameter increased or decreased in each study period relative to the other study periods. Since 14% of the post-dose PK samples were found to be below the lower limit of quantification (LLOQ), the M3 method was applied to limit the potential bias in parameter estimates introduced by LLOQ observations. [23] Model evaluation and selection occurred based on the objective function value (OFV), parameter estimate precision and plausibility, and for the covariate analysis additionally on reductions in IOV or IIV. Goodness-of-fit plots and visual predictive checks (VPCs) were used to graphically evaluate models. [24] Modelling was performed in NONMEM (version 7.5) [25], aided by Perl-speaks-NONMEM (version 5.0.0) [26] and the Pirana interface (version 3.0.0) [27]. R (version 4.0.3) [28] was used for data processing and visualization of modelling output. Detailed descriptions of modelling procedures, underlying equations and assumptions, as well as the NONMEM code of the final model are provided in the Supplementary materials.

2.9. Ethics approval and study registration

All patients included in this trial received pantoprazole and TTM as part of their routine treatment. Ethical approval for this study was provided by the independent Ethics Committee of the Medical University of Vienna with the EK-number 1571/2018. Furthermore, the study was registered in the EUDRACT database with the identifier EUDRACT 2018–002226–22.

3. Results

3.1. Study population

A total of 16 patients were enrolled, all of whom completed P1 and P2. Five patients died during their ICU stay. One patient survived, but had a poor neurological outcome and was not able to give informed consent. Ten patients survived with good neurological outcomes (cerebral performance category 1 or 2) and gave informed consent to a subsequent P3. Thus, for the overall comparison of the three study periods only 10 patients were available. All patients had a presumed cardiac cause of cardiac arrest and underwent coronary angiography (PCI in 8 patients). None of the patients required ECMO, balloon pumps or renal replacement therapy (except for one patient not included in the primary analysis). None of the patients developed acute liver failure. However, for reasons of completeness, individual data, as well as the results of non-compartmental PK of P1 and P2 including all 16 patients are presented in the supplement. The baseline characteristics are presented in Table 2. Genotype analysis of CYP2C19 identified three patients as intermediate, two patients as extensive, four as rapid and one patient as an ultra-rapid metabolizer. Target temperature was reached a median of 92 min (IQR 50–189 min) after admission. The median interval between completed P1 (hypothermic) and the start of P2 (normothermic) was 0.8 days (IQR 0.6–0.9) and the median interval between completed P2 and the start of P3 (conscious) was 8 days (IQR 7–12). Inflammatory parameters were substantially higher in P2 compared to P1 and P3. The median SOFA score was 8 (IQR 7.8–10) at the start of P1, and 7.5 (IQR 7—9) at the start of P2.

4. Pharmacokinetics

Non-compartmental PK showed statistically significant differences in half-life, AUC, Vd and CL of pantoprazole between all study periods (Table 1). The analysis indicated a longer half-life and an increased AUC in P1 and P2 compared to P3. CL was significantly lower in P1 and P2 compared to P3 and the Vd was statistically larger in P2 compared to P1 and P3. Fig. 1 shows concentration-time curves for each study period. Individual data and an exploratory analysis of patients completing P1 and P2 ($n = 16$) are presented in the supplement. The drug concentration-time curves according to metabolizer status are shown in Fig. 2. Due to the small numbers of patients in each subgroup we did not perform inferential statistics, but only presented descriptive statistics (Table 3).

The population PK model confirmed the observations of non-compartmental analysis. PK was best described using a two-compartment model with IIV on CL, and IOV on CL and Vc. In the final model, we estimated CL separately for each study period, indicating that pantoprazole CL was 40% and 29% lower in P1 and P2 compared to P3, respectively. We also found that Vc was 64% higher in P2 compared to the other study periods (Table 4). A parallel covariate analysis using biological variables demonstrated a positive proportional effect of CRP levels on Vc and a negative effect of reduced body temperature on CL. These were best described with a power function. However, these covariates were not included as the reliability of the predicted relationships was impaired by the limited spread in covariate

Table 1
Non-compartmental pharmacokinetic analysis.

	Hypothermia Period 1 (P1)	Normothermia Period 2 (P2)	Recovery period after regaining consciousness Period 3 (P3)	p-value	
Half-life, h, median (IQR)	2.4 (1.8–4.8)	2.8 (2.1–6.8)	1.2 (0.9–2.3)	Overall	$p < 0.001$
				P1 vs P2	$p = 0.046$
				P2 vs P3	$p = 0.005$
				P1 vs P3	$p = 0.007$
Area under the curves, ng/mL*h, median (IQR)	12.7 (9.6–22.6)	9.8 (7.6–18.6)	7.2 (5.9–9.5)	Overall	$p = 0.027$
				P1 vs P2	n.s.
				P2 vs P3	$p = 0.047$
				P1 vs P3	$p = 0.012$
Volume of distribution, Litre median (IQR)	11.4 (10.2–12.7)	16.5 (14.3–21.2)	12.5 (8.0–15.6)	Overall	$p = 0.003$
				P1 vs P2	$p = 0.001$
				P2 vs P3	$p = 0.017$
				P1 vs P3	n.s.
Clearance, l/h median (IQR)	2.9 (1.7–4.0)	3.9 (2.0–4.9)	5.5 (3.9–6.5)	Overall	$p = 0.027$
				P1 vs P2	n.s.
				P2 vs P3	$p = 0.028$
				P1 vs P3	$p = 0.047$

QR: interquartile ranges; n.s.: not significant

Table 2
Baseline characteristics.

	Admission/Baseline Characteristics	Hypothermia	Normothermia	Recovery period after regaining consciousness
Age, median (IQR)	53 (46–62)	–	–	–
Body mass index, median (IQR)	28 (24–34)	–	–	–
Female, n (%)	3 (30)	–	–	–
Body temperature °C, median (IQR), at the start of the period	35.8 (35.1–35.9)	33.1 (32.5–33.4)	36.5 (36.2–37.2)	36.4 (35.9–36.8)
Body temperature °C, mean (SD), during the period	–	32.9 (0.41)	36.9 (0.53)	36.4 (0.38)
Minutes to target temperature after admission, median (IQR)	–	92 (50–189)	–	–
SOFA-Score, median (IQR), at the start of the period	n.a.	8 (7.8–10)	7.5 (7–9)	n.a.
Laboratory results, median (IQR)				
Leukocytes, [4–10 * 10^9/L]	14.5 (8.5–23.7)	–	9.8 (7.0–12.0)	9.0 (7.1–11.9)
Platelets, G/L [150–350 * 10^9/L]	239 (207–316)	–	189 (132–231)	428 (301–556)
Hemoglobin, g/dL [13.5–18.0 g/dL]	13.5 (8.5–23.7)	–	13.1 (10.8–13.6)	11.8 (10.7–12.7)
Creatinine, mg/dL [0.7–1.20 mg/dL]	1.02 (0.83–1.25)	–	0.69 (0.46–0.90)	0.75 (0.66–1.07)
ASAT, U/L [< 50 U/L]	138 (99–164)	–	101 (69–169)	35 (23–46)
ALAT, U/L [< 50 U/L]	119 (76–201)	–	110 (66–139)	43 (31–71)
γ-GT, U/L [< 60 U/L]	60 (42–150)	–	44 (33–119)	92 (60–191)
Fibrinogen, mg/dL [200–400 mg/dL]	244 (228–286)	–	400 (327–425)	503 (467–597)
Prothrombin time, % [70–125%]	77 (59–112)	–	78 (63–101)	87 (75–96)
Albumin, g/L [35–52 g/L]	36 (34–43)	–	32 (28–33)	35 (34–38)
Cholinesterase, kU/L [3.65–12.92 kU/L]	7.31 (6.34–8.09)	–	6.51 (5.62–7.05)	6.55 (5.73–7.67)
CRP, mg/dL [< 0.5 mg/dL)	0.13 (0.04–0.39)	–	9.15 (5.86–13.5)	1.39 (0.96–2.21)
IL-6, pg/mL [< 7 pg/mL]	21.7 (14.4–76.1)	–	n.a	n.a
Procalcitonin, ng/mL [< 0.5 ng/mL)	0.04 (0.03–0.05)	–	n.a	n.a
Medical history n (%)				
Diabetes	2 (20)	–	–	–
Coronary heart disease	2 (20)	–	–	–
Hypertension	4 (40)	–	–	–
Alcohol abuse	1 (10)	–	–	–
Hyperlipidemia	3 (30)	–	–	–
Obesity	2 (20)	–	–	–
Hypothyroidism	1 (10)	–	–	–
Resuscitation factors		–	–	–
Witnessed collapse, n (%)	10 (100)	–	–	–
Bystander resuscitation, n (%)	10 (100)	–	–	–
Initial shockable rhythm, n (%)	10 (100)	–	–	–
Time to ROSC, minutes mean (SD)	16 (9,5)	–	–	–
Underwent coronary angiography, n (%)	10 (100)			
PCI, n%	8 (80)			

SOFA Score includes: pO2/Fi02, Catecholamines, Glasgow coma scale, bilirubine, platelets, creatinine; ASAT: aspartat-aminotransferase, ALAT:alanine amino-transferase, γ-GT: gamma-glutamyl transferase, CRP: C reactive protein, IL-6: Interleukin 6, ROSC: Return of spontaneous circulation, PCI: percutaneous coronary intervention;

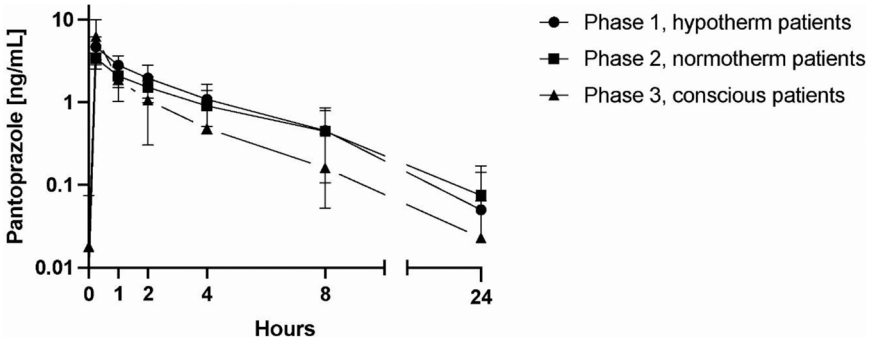

Fig. 1. Pantoprazole time-concentration curves. shows pantoprazole time-concentration-curves for the 3 study periods (hypothermic = P1, normothermic = P2 and conscious = P3, each n = 10) in ng/mL. Means ± standard deviations are presented. The area under the time-concentration-curve (AUC) differed significantly between the three periods (p = 0.027, for overall comparison, P1 vs. P2 non-significant, P1 vs. P3 p = 0.012, P2 vs. P3 p = 0.047). Other non-compartmental pharmacokinetic parameters are presented in Table 1.

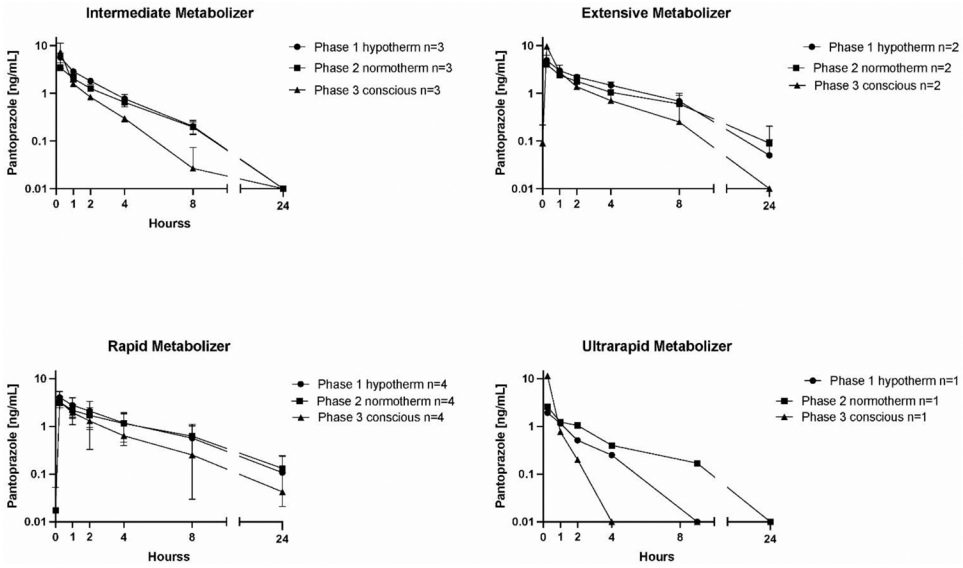

Fig. 2. Pantoprazole time-concentration curve according the metabolizer status shows pantoprazole time-concentration curves stratified by metabolizer status. 10 patients completed the 3 study periods, of these 3 patients were intermediate metabolizers, 2 were extensive metabolizers, 4 were rapid metabolizers and 1 was an ultrarapid metabolizer. Means and standard deviations are presented. Due to the small number of patients in each metabolizer group we refrained from calculating inferential statistics, but present descriptive statistics for informative purposes in Table 3.

values (Fig. S1). The model describes the data well and has good predictive performance as can be seen in the VPCs (Fig. 3) and the plots showing predicted versus observed concentrations (Fig. S3). It should be noted that the model overpredicts the observations of the only ultrarapid metabolizer in the study population, which was exacerbated by the estimation of separate, lower values for CL in P1 and P2 in the final model (Fig. S2). Detailed results of the structural and covariate model development, including a discussion and comparison of the two parallel covariate analyses, plots showing IIV and IOV versus tested covariates, and observed versus predicted concentration plots are provided in the supplementary population PK analysis results.

5. Discussion

The main findings of this study were an impaired pantoprazole metabolism (i) during TTM at 33 °C (P1) and (ii) after rewarming in the ICU (P2) when compared to the internal control period (P3, the "conscious" period) and to published data [29]. Different contributing mechanisms may be identified: In P1 the main effect can be attributed to a ~40% reduced clearance, while in P2 a combination of a reduced clearance (~29%) and an increased Vc (~64%) was observed.

Our data do not allow for the ultimate identification of the underlying mechanisms for these results. However, the impact of pro-inflammatory cytokines and systemic inflammation on the activity of CYP enzymes and consequently on the PK of pantoprazole was previously shown and therefore to be expected in P2. [12,13] In line with other studies, all patients in this study had elevated interleukin-6 levels on admission (median 21.7 pg/mL) and consecutively increased CRP levels (median 10.7 mg/dL) in P2. [30,31] Interestingly, Shedlovsky et al. used human endotoxemia trials to demonstrate that the effect of pro-inflammatory cytokines on CYP enzyme activity is not immediate, but that it evolves over a 24-hour period. [32]. Likewise, Tortorici et al. demonstrated a downregulation of CYP3A2 in a rat model of cardiac arrest only after 24 h [33]. Interestingly, TTM and IL-6 blockade were able to abolish this effect, emphasizing potentially positive effects of TTM on post-cardiac arrest syndrome and the central role of IL-6. Experimental data also confirms that the Vd is increased during systemic inflammatory responses, presumably by increased vascular permeability causing rapid hypoalbuminemia and edema formation, which was also shown to be a hallmark of post-cardiac arrest syndrome [34,35]. In our study, target temperature was reached within 2 h of hospital admission. Thus, systemic inflammation is unlikely to be responsible for the PK alterations observed during the beginning of P1. [32,33] These data rather suggest that hypothermia has an immediate effect on CYP-mediated drug metabolism. One possible explanation could be that TTM forces cytochrome enzymes to work outside of their optimum temperature range. [36] However, it is important to note that during a 24 h-period the effects of decreased body temperature and systemic inflammatory response may not be fully separable, but rather coalesce over time. Nee et al. recently published a very interesting study which investigated liver function after cardiac arrest and hypothermia using the LiMAx test (in short, a test to assess CYP1A2 activity by measuring $^{13}CO_2$ in exhaled air after i.v. administration of ^{13}C-methacetin). Similar to our findings, they demonstrated a reduced CYP1A2 activity on the first day following cardiac arrest, while C-reactive protein concentrations were not elevated. The observed effect was most

Table 3
Pharmacokinetics according the CYP-2C19 metabolizer state.

	Overall	* 1/* 2	* 1/* 1	* 1/* 17	* 17/* 17
Pharmacokinetics	n = 10	n = 3	n = 2	n = 4	n = 1
Half-life, h median (IQR)					
Hypothermic patients (period 1)	2.4 (1.8–4.8)	1.9 (1.8–2.1)	3.8 (2.8–4.8)	4.3 (2.2–6.4)	1.4
Normothermic patients (period 2)	2.8 (2.1–6.8)	2.1 (1.9–2.7)	5 (2.5–7.5)	5.8 (3.2–7.2)	3
Conscious patients (period 3)	1.2 (0.9–2.3)	1.3 (1.2–1.8)	1.9 (0.8–2.9)	1.6 (0.9–4.2)	0.7
Area under the curves, ng/mL*h median (IQR)					
Hypothermic patients (period 1)	12.7 (9.6–22.6)	9.7 (9.6–13.0)	18.2 (13.8–22.6)	17.7 (9.6–27.7)	3
Normothermic patients (period 2)	9.8 (7.6–18.6)	7.7 (7.6–8.0)	15.1 (11.6–18.6)	19.1 (11.2–22.3)	5.5
Conscious patients (period 3)	7.2 (5.9–9.5)	6.1 (5.9–8.6)	9.6 (7.7–11.5)	6.5 (3.3–15.4)	6.7
Volume of distribution, Litre median (IQR)					
Hypothermic patients (period 1)	11.4 (10.2–12.7)	10.1 (8.7–10.7)	11 (10.2–11.9)	12.1 (11.4–13.2)	21.3
Normothermic patients (period 2)	16.5 (14.3–21.2)	14.3 (13.4–18.0)	16.2 (11.2–21.2)	17.5 (14.7–21.1)	22.5
Conscious patients (period 3)	12.5 (8.0–15.6)	10.6 (8.0–16.8)	9.2 (6.1–12.3)	15.3 (13.8–15.7)	6.1
Clearance, l/h median (IQR)					
Hypothermic patients (period 1)	2.9 (1.7 −4.0)	4 (2.9–4.0)	2.1 (1.7–2.6)	2.2 (1.4–4.3)	11.6
Normothermic patients (period 2)	3.9 (2.0–4.9)	4.7 (4.7–4.9)	2.5 (2.0–3.1)	2 (1.6–5.7)	6.6
Conscious patients (period 3)	5.5 (3.9–6.6)	6.1 (4.4–6.6)	4 (3.0–5.1)	7.5 (2.8–11.8)	5.9

QR: interquartile range; * 1/* 2 intermediate metabolizer, * 1/* 1 extensive metabolizer, * 1/* 17 rapid metabolizer, * 17/* 17 ultra-rapid metabolizer

Table 4
Parameter estimates of the final model.

Description	Parameter	Units	Estimate	RSE (%)[b]
Structural model[a]				
Clearance	θ_{CL}	L/h	4.83	15.8
Central distribution volume	θ_{Vc}	L	7.22	11.6
Peripheral distribution volume	θ_{Vp}	L	3.25	12.3
Inter-compartmental clearance	θ_{Q}	L/h	1.91	22
Inter-individual variability				
Clearance	$\omega_{CL,IIV}$	CV%[c]	46.8	17.3
Inter-occasion variability				
Clearance	$\omega_{CL,IOV}$	CV%	31.5	27.1
Central distribution volume	$\omega_{Vc,IOV}$	CV%	48.2	19.1
Residual variability				
Additive error (SD)	σ_{add}	mg/L	0.0381	20.4
Proportional error	σ_{prop}	CV%	12.3	13
Covariate model				
Factor P1 on CL	$\theta_{CL\text{-}P1}$	–	0.598	17.2
Factor P2 on CL	$\theta_{CL\text{-}P2}$	–	0.709	17.2
Factor P2 on Vc	$\theta_{Vc\text{-}P2}$	–	1.64	11.2
Allometric scaling: CL, Q	$\theta_{allo,CL,Q}$	–	0.75 FIX	–
Allometric scaling: Vc, Vp	$\theta_{allo,Vc,Vp}$	–	1 FIX	–

[a] All PK parameters were allometrically scaled according to: $P = \theta_P \times \left(\frac{WT}{90}\right)^{\theta_{allo}}$, with θ_P being the typical value of structural model parameter *P* for an individual with the median body weight in the study population (90 kg), *WT* being patient body weight, and θ_{allo} being the allometric exponent; [b] RSE, relative standard error, calculated as *SE/parameter estimate* × 100%, and for IIV and IOV as $(SE/\omega^2)/2 \times$ 100%; [c] CV%, coefficient of variation, calculated according to $\sqrt{e^{\omega^2}-1} \times 100\%$; SD, standard deviation.

prominent on day 2 after rewarming, when biomarkers of systemic inflammation were also upregulated, and showed a slow recovery until day 10. [37].

The findings of this study have important implications on the choice of drugs during TTM. Pantoprazole was used as a probe drug for CYP2C19-dependent metabolism and the results may be extrapolated to many other drugs. [6] For example, in patients with acute coronary syndromes complicated by cardiac arrest, TTM is initiated before or during coronary catheterization. Clopidogrel and also prasugrel have been shown to have an attenuated platelet inhibition during TTM, which may be caused by an impaired drug absorption, but also by an impaired activation of the prodrugs. [38,39] While in healthy volunteers the concentration of clopidogrel active metabolite exceeds the concentration of the prodrug approximately 30-fold, in patients after cardiac arrest treated with TTM this ratio was < 1. [40,41] Furthermore, drugs such as morphine, fentanyl or midazolam which are regularly used for the analgosedation of cardiac arrest patients also showed a reduced clearance. [5] Additionally, Baldwin et al. showed that the half-life of fentanyl, which is metabolized by CYP3A4, is significantly longer in patients after cardiac arrest undergoing TTM compared to healthy controls, but not compared to an ICU population. This study examined plasma levels 12 h after cessation of continuous administration and compared them to expected levels in historical control groups [42]. Interestingly, although fentanyl is metabolized via CYP3A4, they observed a 46% reduced clearance, which is comparable to our data (−40% in P1). Possible implications of these findings for clinicians include a prolonged awakening period in sedated patients, an insufficient platelet inhibition by clopidogrel and potentially prasugrel due to insufficient metabolic activation of pro-drugs, and toxic effects of CYP450 mediated drugs.

All patients included were genotyped for CYP2C19 polymorphisms. However, we didn't power the study for the inclusion of a certain number of patients per polymorphism. Only one ultra-rapid metabolizer was included. Expectedly, the PK of this patient differed substantially from other patients in all three study periods (Table 3) [11] and the population PK model overpredicts the observations for this patient (Fig. S2). The included sample size limits generalizations based on our findings. The observed heterogeneity of PK between patients in all three periods could prove useful for the design of future trials involving CYP-dependent drugs.

TTM is not only performed in patients who have undergone successful CPR, but is also routine in certain surgeries and was already tested for patients suffering myocardial infarction, stroke and

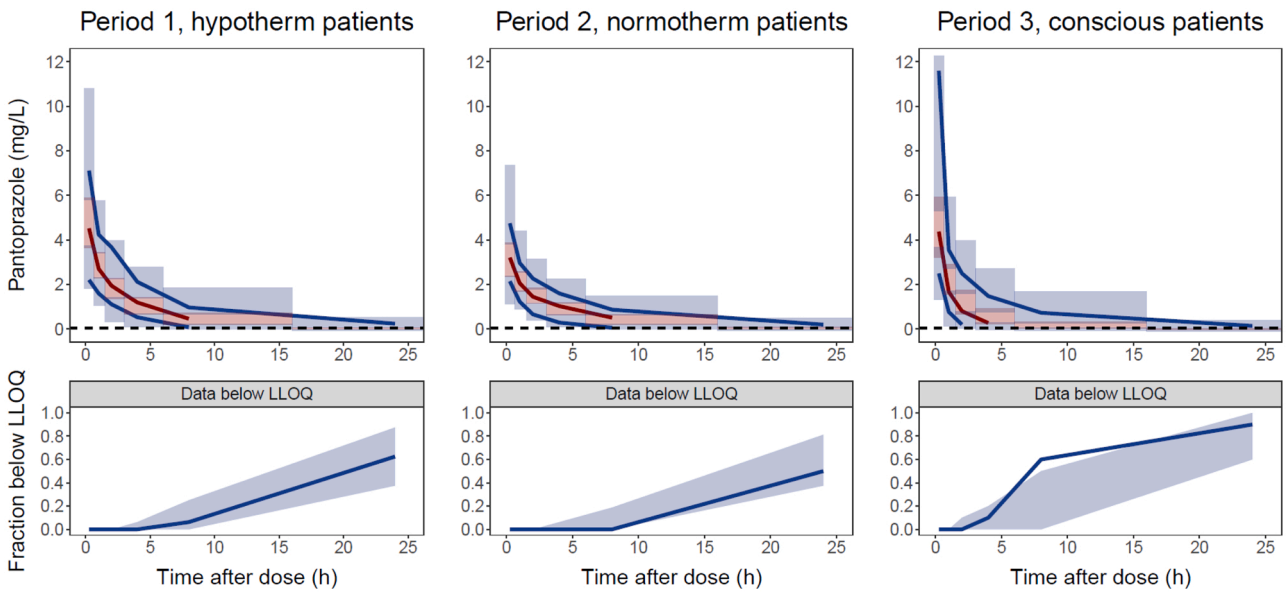

Fig. 3. Visual predictive checks (VPC) of the pantoprazole concentration-time profile stratified by study period. The calculations were performed based on 10 patients who completed all three study periods. In the upper panels, the solid lines represent the median (red) and 5th and 95th percentiles (blue) of the observed data. The shaded areas represent the 95% confidence intervals around the median and 5th and 95th percentiles of the simulated data. The dashed black line indicates the lower limit of quantification (LLOQ). The lower panels in each plot are the visual predictive checks (VPC) for the data below the LLOQ. The solid line indicates the observed fraction of data below the LLOQ. The shaded areas represent the 95% confidence intervals around the simulated median fraction of data below the LLOQ.

intracerebral hemorrhage. [43–45] Furthermore, there are still ongoing trials, for example in acute myocardial infarction (NCT01777750), brain injury (NCT02996266) or stroke (NCT01833312). Thus, TTM may be used increasingly in the treatment of patients with various diseases. For instance, therapeutic hypothermia is routinely applied in neonates with hypoxic/asphyxia encephalopathy. In this patient collective, a decreased Cl of various antibiotics has been shown.[46–48].

In general, there is a trend towards personalized medicine, which is in contrast with the "one-size-fits-all" principle of drug dosage. Still, therapeutic drug monitoring is hardly applied during TTM, but could improve treatment and reduce dangerous side effects. Furthermore, pharmacokinetic or pharmacodynamic data in vulnerable populations are frequently lacking and drug doses are seldom based on scientific data obtained in that respective population, but rather are frequently extrapolated from other patient populations with massively differing pathophysiological properties. While the influences of genetic polymorphism in drug metabolizing enzymes are widely accepted, the clinical situation of each patient, including factors such as systemic inflammation or body temperature which may cause so called “phenoconversion” is mostly neglected. In that respect, our data improve understanding of PK to optimize treatment for this especially vulnerable patient population, which is regularly omitted from PK studies.

The particular strength of this trial is the longitudinal trial design with repeated inclusion of the same patients in up to three study periods, which allows for intra-individual comparison of PK and overcomes important limitations of other studies in this field.

In addition to the described strengths, some limitations must be considered. Due to the inclusion criterion "expected life expectancy > 3 days", patients with a presumably better prognosis than the average cardiac arrest population were included. This assessment was conducted by the attending emergency physicians and the study team based on individual resuscitation characteristics (e.g., witnessed cardiac arrest, short low-flowtime, shockable rhythm, etc.). Thus, none of the ten patients who completed all three study periods developed acute renal or hepatic failure. Such complications frequently occur following cardiac arrest and naturally may impact on drug metabolism. At our center, TTM at 33 °C is performed in all patients (except if an absolute contraindication exists). Therefore, our results are only applicable for patients treated at this target temperature. In the “conscious” study period not all laboratory parameters (CRP in particular) were within normal ranges. Thus, these PK data were not obtained from an entirely healthy population and a slightly impaired drug metabolism compared to a completely healthy population is likely. For instance, Gawrońska-Szklarz et al. reported a pantoprazole half-life of 1.27 h ± 0.41 h in healthy extensive metabolizers (CYP2C19 *1/*1), which is shorter than that demonstrated by our population (Table 3). [11] Nonetheless, in the “conscious” period, patients were still in hospital and much longer time intervals would have been necessary to ensure a completely normal drug metabolism. This would inevitably have caused organizational issues. This study excluded patients receiving potent CYP450 inducers or inhibitors, but it did not otherwise interfere with the regular treatment of patients in intensive care units or on normal wards, and therefore concomitant intake of other CYP450 metabolized drugs and potentially of weak inducers or inhibitors was possible. An optimal control group to prove causality would be patients after cardiac arrest without TTM. However, a hypothermia study in rats convincingly showed that a decrease in body temperature from 37° to 32°C decreased the metabolic clearance of a probe by about 60% and doubled its AUC [49]. This effect size is comparable to what we observed in the current trial and makes it likely that TTM was at least in part responsible for the observed increase in half-life and AUC and reduced clearance in our patient population.

6. Conclusion

Targeted temperature management had an immediate impact on drug metabolism. These results have important implications for the choice of drugs and the optimal treatment of patients undergoing TTM and may provide inspiration for further studies in similar situations.

CRediT authorship contribution statement

MP, CS and CW designed the study. CC, CS, MM, and MR participated in data acquisition. HH, MS and BJ analysed the data and conducted methodology inputs. BR and RS-P conducted laboratory analyses. CS, WvO and JGCvH performed statistical analyses and analysed the data. MP and CS interpreted the data and wrote the manuscript. CW, CS, BJ,

MS and revised the manuscript for important intellectual content. All the authors reviewed the final draft of the manuscript and MP and CS approved the final version to be published on Critical Care. All authors read and approved the final manuscript.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2021.112573.

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