Effects of hypothermia on the disposition of morphine, midazolam, fentanyl, and propofol in intensive care unit patients

Thor Wilhelm Bjelland, Pål Klepstad, Bjørn Olav Haugen, Turid Nilsen, Ola Dale

The laboratory of origin resides in the Dept. of Circulation and Medical Imaging, Faculty of medicine, NTNU, Trondheim, Norway

Dept of Circulation and Medical Imaging, Faculty of medicine, NTNU, Trondheim, Norway (TWB, PK, BOH, TN, OD).

Dept of Anesthesiology and Emergency Medicine, St. Olav's University Hospital, Trondheim, Norway (PK, OD).

Dept of Cardiology, St. Olav's University Hospital, Trondheim, Norway (BOH).
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Corresponding author: Thor Wilhelm Bjelland
Address: NTNU, Det medisinske fakultet, Institutt for sirkulasjon og bildediagnostikk, Postboks 8905, 7491 Trondheim, Norway
E-mail: thor.w.bjelland@ntnu.no

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Non-standard abbreviations:

AUC\textsubscript{sample} - area under curve in the sample period, BMI - body mass index, Cals - calibration standards, Cl\textsubscript{tot} - total clearance, Cl\textsubscript{R} - renal elimination clearance, C\textsubscript{SS} - steady state concentration, CPC - cerebral performance category, CPR - cardiopulmonary resuscitation, CYP3A4 - Cytochrome P450 3A4, GCMS - gas chromatography mass spectrometry, GCS - Glasgow coma scale, ICU - intensive care unit, IS - internal standard, λ\textsubscript{z} - elimination rate constant, LCMS/MS - liquid chromatography and tandem
mass detection, LOQ - limit of quantification, M3G - morphine-3-glucuronide, M6G - morphine-6-glucuronide, MAAS - motor activity assessment scale, MAP - mean arterial pressure, MP - mobile phase, OH-midazolam - α-hydroxy-midazolam, PK - pharmacokinetic, PT-INR - prothrombin time - international normalized ratio, Q - qualifier ion, QCs - quality controls, RT - retention time, SAPS II - simplified acute physiology score II, T₀ - the start of the pharmacokinetic study period, TH - therapeutic hypothermia, UGT2B7 - uridine diphosphate glucuronosyltransferase 2B7
Abstract

Therapeutic hypothermia may induce pharmacokinetic changes that may affect the level of sedation. We have compared the disposition of morphine, midazolam, fentanyl, and propofol in therapeutic hypothermia with normothermia in man. METHODS: Fourteen patients treated with therapeutic hypothermia (TH) following cardiac arrest (33-34°C) were compared with 8 matched critically ill patients (36-38°C). Continuous infusions of morphine and midazolam were stopped and replaced with infusions of fentanyl and propofol to describe elimination and start of infusion pharmacokinetics, respectively. Serial serum and urine samples were collected for 6-8 hours for validated quantification and subsequent pharmacokinetic analysis. RESULTS: During TH, morphine elimination half-life (T$_{1/2}$) was significantly higher, while total clearance (Cl$_{tot}$) was significantly lower (median [semi-iqr]): T$_{1/2}$: 266 [43] vs 168 [11] min, p<0.01, Cl$_{tot}$ 1201 [283] vs 1687 [200] ml/min, p<0.01. No significant differences were seen for midazolam. Cl$_{tot}$ of fentanyl and propofol was significantly lower in hypothermic patients (median [semi-iqr]: Fentanyl 726 [230] vs 1331 [678] ml/min, p<0.05, propofol: 2046 [305] vs 2665 [223] ml/min, p<0.05). CONCLUSION: Compared to the matched, normothermic ICU patients, elimination half-life of morphine was significantly higher during TH. Total clearance was lower during TH for morphine, fentanyl, and propofol, but not for midazolam. Reducing the infusion rates of morphine, fentanyl, and propofol during therapeutic hypothermia is encouraged.
Introduction

Two pivotal studies have established the efficacy of treating comatose survivors of cardiac arrest with therapeutic hypothermia (TH) (33-34°C for 12 to 24 hours) (Bernard et al., 2002; Peberdy et al., 2010; The Hypothermia After Cardiac Arrest Study Group, 2002). Patients treated with TH are given sedatives and analgesics in order to tolerate mechanical ventilation and to avoid shivering. Continuous infusions of morphine, fentanyl, midazolam, and propofol are among the most commonly used drugs for analgesia and sedation at the intensive care unit (ICU) (Payen et al., 2007).

Hypothermia can induce significant physiological changes that affect drug disposition and action through changes in both metabolism and distribution (Alcaraz et al., 1989; Arpino and Greer, 2008; Asokumar et al., 1998; Bansinath et al., 1988; Beaufort et al., 1995; Caldwell et al., 2000; Fukuoka et al., 2004; Kadar et al., 1982; Koren et al., 1987; Leslie et al., 1995; Polderman, Kees H., 2009; Tortorici et al., 2007). Reduced metabolism due to changes in enzyme activity during hypothermia may increase drug serum levels, and thus drug effects and duration of action (Arpino and Greer, 2008; Polderman, K. H., 2004). However, the effect of hypothermia on the activity of different enzymes vary; while 10°C lower temperature reduced CYP P450 activity on diazepam by 22%, conjugation of oxazepam was reduced by only 14% (Mortensen and Dale, 1995). In addition, while CYP3A and CYP2E activity as measured by clearance of midazolam and chlorzoxazone was approximately 50 and 60% lower in rats given CA and TH compared with the control group, respectively, no differences were demonstrated for CYP2C/D (Zhou et al., 2011). This substrate specificity implies that effects of hypothermia on PK
Morphine is metabolized by UGT2B7, but to our knowledge, no studies on the effect of hypothermia on isolated UGT2B7 exist. In animals, reduced glucuronidation and increased serum concentrations are consistent with results from neonates, who had approximately 40% higher serum concentrations and 23% reduced clearance at 33-34°C compared to normothermic controls (Bansinath et al., 1988; Rink et al., 1956; Róka et al., 2008). CYP3A4 metabolizes both midazolam and fentanyl. A systematic review found CYP P450 activity was reduced between 7 and 22% per degree Celsius below 37°C (Tortorici et al., 2007). In vitro, CYP3A activity was reduced to 69±1% at 32°C, and at 33°C the maximal enzymatic velocity of midazolam was reduced by approximately 13% (Empey et al., 2011; Fritz et al., 2005). Elimination of fentanyl was essentially stopped in children cooled to 18-25°C (Koren et al., 1987). The effect of hypothermia on clearance of midazolam in humans has been reported to vary from naught to more than 100-fold, and an 11.1% reduction in clearance per degree of reduced core temperature was estimated (Fukuoka et al., 2004; Hostler et al., 2010). The metabolism of propofol in humans depends on liver flow and involves several enzymes such as UGT1A8/9, CYP2C9, and CYP2B6. No significant differences in the activity of CYP2C were seen in a study on rats, but the clearance of phenytoin (CYP2C9 and CYP2C19) was reduced by 67% (Iida et al., 2001; Zhou et al., 2011). Propofol blood concentrations were increased by approximately 20% both in healthy volunteers at 34°C and during hypothermic
cardiopulmonary bypass, likely due to reduced intercompartmental clearances in the former (Leslie et al., 1995; Russell et al., 1989).

In summary, reduced elimination during hypothermia has been showed for morphine, midazolam, and fentanyl both in animals and in man (Bansinath et al., 1988; Fritz et al., 2005; Fukuoka et al., 2004; Koren et al., 1987). Study results are less uniform regarding apparent volume of distribution, where it was reported increased for midazolam and reduced for morphine and fentanyl (Bansinath et al., 1988; Fukuoka et al., 2004; Koren et al., 1987). However, these studies were performed in vitro, animals, children, or healthy volunteers, settings that limit their validity with regard to adult patients treated with TH after cardiac arrest. However, the existing evidence suggest that hypothermia can induce clinically significant pharmacokinetic (PK) changes increasing the risk of detrimental over sedation, thus more knowledge on drug disposition during TH is needed (Bjelland et al., 2010; Koren et al., 1987; Pedersen et al., 2007; Polderman, K. H., 2004; Tortorici et al., 2007). We have therefore explored the disposition of morphine, midazolam, fentanyl, and propofol during TH following cardiac arrest in man.
Materials and Methods

Ethics and approvals

This study was approved by the Regional Committee for Medical Research Ethics and The Norwegian Medicines Agency. Next of kin were informed and accepted participation on behalf of the patient before inclusion. Deferred informed consent was obtained from the patients with an adequate cerebral outcome, defined as Cerebral Performance Category (CPC) 1 or 2, 1-4 weeks after intervention.

Setting

Patients treated with TH were recruited at St. Olav's University Hospital in Trondheim, a tertiary university hospital with a catchment population of 650,000.

Design

This was a prospective, case-control trial. Patients treated with TH were compared with a matched group of critically ill normothermic patients (controls). Patients under treatment with TH in the General ICU or the Coronary Care Unit were assessed for eligibility. The indications for TH during the study period were comatose patients following successful CPR. Inclusion criteria were adult (age ≥ 18 years) patients in need of continuous analgesia and sedation with morphine and midazolam, and mechanical ventilation, for at least 12 hours. Patients with known renal failure or clinically significant liver failure, known history of substance or alcohol abuse, pregnancy, severe cardiovascular instability (i.e. recurrent cardiac arrests), hemoglobin < 11 g/dl, and history of allergies for the study
drugs were excluded. Controls were normothermic (36-38°C) intensive care patients matched on sex, age, and duration of morphine infusion.

Therapeutic procedures

TH was established immediately after admission as follows: Active external cooling (Thermowrap Universal or CureWrap cooling blanket with Allon or CritiCool control unit, respectively, both by MTRE Advanced Technologies Ltd) was started when the patient was sedated and on controlled ventilation. The target was to maintain rectal temperature at 33(±1)˚C for 12-24 hours. Neuromuscular blockade (atracurium) was administered during cooling and rewarming to eliminate shivering. Serum electrolytes and glucose were monitored and kept within normal ranges. Administration of 30 ml/kg Ringer’s Acetate solution (4˚C) was recommended within an hour of admission. Target mean arterial pressure (MAP) was 70-100 mmHg, and hypotension was treated with crystalloid fluids and vasopressor agents (norepinephrine, dopamine). Target diuresis was 1 ml/kg/hour. Seizures were controlled with sedatives and/or anticonvulsants.

Study drugs were administered by continuous i.v. infusion according to the standard procedures. Study drugs were: morphine (Nycomed Pharma AS, Norway) 5mg/ml, and midazolam (Alpharma AS, Norway, or F. Hoffmann-La Roche AG, Switzerland) 5mg/ml. Intensive care nurses and the attending physician titrated doses to maintain a Motor activity assessment scale-score (MAAS-score) of 0-1, and to avoid shivering (Devlin et al., 1999). During infusions of neuromuscular blocking agents, sedatives and analgesics were given in doses decided by the attending physician to ensure adequate
sedation to avoid awareness. Other therapeutic procedures related to the patients’ clinical condition were at the discretion of the attending physician.

T₀ expressed the start of the PK study period. Patients received continuous morphine and midazolam infusions for at least 7.5 h before T₀. Patient core temperature was below 34°C for at least two hours before T₀. At T₀ the infusions of morphine and midazolam were stopped, and replaced by infusions of fentanyl 0.05 mg/ml (Alpharma AS, Norway) and propofol 10 mg/ml (Alpharma AS, Norway). Fentanyl was administered in a dose corresponding to 1/100 of the titrated morphine infusion and propofol was started at dose of 1-2 mg/kg/h (Fig. 1).

Recordings

Sex, age, weight, height, ethnicity, and simplified acute physiology score II (SAPS-II) scores at the time of hospital admission and for the first 24 hours were recorded. Because TH was only given to comatose survivors of cardiac arrest, Glasgow Coma Scale (GCS) was low for all patients in the hypothermic group. To compare severity, SAPS-II scores were therefore calculated without GCS, but otherwise as reported by Le Gall et al. (Le Gall et al., 1993). Current medications and infusion rates of study drugs were retrieved from the medical records and infusion pumps. Total serum concentration of bilirubin, albumin, and creatinine, and prothrombin time - international normalized ratio (PT-INR) were analyzed using standard clinical chemistry methods. MAAS-scores, intra-arterial blood pressure, heart rate, urine production, and rectal temperature were recorded at the each blood sampling point. Finally, the amount of creatinine excreted during the
collection interval (concentration * volume) was divided by s-creatinine to calculate creatinine clearance.

Drug analysis

Blood samples were collected from an indwelling arterial cannula at 0, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 300 and 360 minutes after T0 in Vacuette tubes (Greiner Bio One, Austria) without additives. Hourly urine samples were collected at 0, 60, 120, 180, 240, 300 and 360 minutes. Two additional samples of blood and urine were collected at 420 and 480 minutes if hypothermia still lasted. All blood samples were centrifuged for 10 minutes at 1400g, with additional 3-5 minutes of centrifugation in cases of unsuccessful separation. After transfer to cryotubes, serum was immediately cooled to 0°C, frozen at -20°C within three hours, and stored in a -80°C freezer within a week until analysis.

Serum concentrations were quantified with fully pre- and in-run validated methods according to the criteria described by Shah, Dadgar and co-authors. (Dadgar et al., 1995; Shah et al., 1991). Quality controls (QCs) and calibration standards (Cals) where prepared in plasma from blood donors (Department of Immunology and Transfusion Medicine, St Olav's Hospital, Trondheim). For urine analysis, quality controls and calibration standards were prepared in urine from a healthy and drug-free volunteer.

Morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) in serum were analyzed essentially according to Tyrefors et al. (Tyrefors et al., 1996) using liquid chromatography and tandem mass detection (LCMSMS) (LC; HP1100 Agilent
Technologies, Palo Alto, CA, USA), MS/MS; API4000, triple quadrupole, (Applied Biosystems/MDS Sciex Instruments, Foster City, CA, USA). Patient samples, Cals and QC's (200 µl serum or plasma) were all spiked with morphine-d3, M3G-d3 and M6G-d3 as IS. For morphine serum and urine analysis, all standard material was from LIPOMED AG (Arlesheim, Switzerland). The samples, Cals and QC's were mixed with 1 ml NH₄ HCO₃ (10mMolar, pH 9.3) and transferred to a solid phase column (SPE, C18, 200 mg, 3 ml Bond Elute from Varian Inc., Lake Forest, CA, USA). The analytes were eluted by methanol and the elution volume evaporated to dryness before reconstituted in 150 ml mobile phase (MP) consisting of 5% acetonitrile in 0.1% formic acid. The analytes in the reconstituted solution (80 µl) were injected to and separated on a Zorbax SB-18 column (5 µm, 150 mm × 4.6 internal diameter from Agilent Technologies Palo Alto, CA, USA). The analytes were quantified by the masses of 286/152 for morphine and 462/286 for M3G and M6G. M3G and M6G were identified by different retention time (RT) (2.20 and 3.37 min for M3G and M6G, respectively). Calibration ranges were 1.0-50.0, 5.0 -500 and 1.0-60 ng/ml for morphine, M3G and M6G, respectively. The concentration of QC's (QC 1-3) was 3 or 6, 50 and 75% of the highest calibration standard for each analyte. The correlation coefficient (r²) were >0.995 for all calibration curves. Limit of quantification (LOQ) with coefficient of variation (CV) <20%, n=18 was 1.0, 5.0 and 1.0 ng/ml for morphine, M3G and M6G, respectively. In the pre-run validation, CV (n=18x3 (18 for each QC level)) for QC's 1-3 was < 6.2, 5.3 and 11.6% for morphine, M3G and M6G, respectively. Inaccuracy (n=18x3) for QC's 1-3 was < 9.0, 11.7 and 6.6% for morphine, M3G and M6G, respectively. In-run, CV (n=14x3) for QC's 1-3 was < 9.5, 8.9
and 8.7% for morphine, M3G and M6G, respectively. Inaccuracy (n=14x3) for QCs 1-3 was < 3.6, 3.8 and 6.5% for morphine, M3G and M6G, respectively.

Urine concentrations of morphine, M3G and M6G were analyzed essentially according to Gustavsson et al. using the same equipment as for serum analysis (Gustavsson et al., 2007). Patient samples, Cals and QCs (20 µl urine) were all diluted with 80 µl H₂O spiked containing d-3 deuterated morphine, M3G and M6G (IS). The diluted samples, Cals and QCs (10 µl) were injected and quantified in the same manner as for morphine. Calibration ranges were 10.0-10000 ng/ml for all analytes. The r² were >0.995 for all calibration curves. LOQ (CV<20%, n=18) was 10.0 ng/ml for all analytes. The concentration of QCs (QC 1-3) was 0.25, 50, and 75% of the highest Cal standard for each analyte. In the pre-run validation, CV (n=18x3) for QCs 1-3 was < 9.4, 14.1 and 9.9% for morphine, M3G and M6G, respectively. Inaccuracy (n=18x3) for QCs 1-3 was < 4.2, 3.6 and 5.0% for morphine, M3G and M6G, respectively. In-run, CV (n=6x3) for QCs 1-3 was < 10.3, 10.1 and 7.4% for morphine, M3G and M6G, respectively. Inaccuracy (n=6x3) for QCs 1-3 was < 9.1, 6.9 and 6.3% for morphine, M3G and M6G, respectively.

Serum concentrations of midazolam and its active metabolite α-OH-midazolam (OH-midazolam) were determined essentially according to Martens et al. (Martens and Banditt, 1997). A Hewlet-Packard (HP) 6890N gas chromatograph and a HP 5975B inert MSD mass spectrometer (GC-MS) were used (Agilent Technologies Inc, Wilmington DE). Patient samples, Cals and QCs (1.0 ml serum or plasma) were all spiked with
midazolam-d4 and OH-midazolam-d4 as IS. All standard material was from Cerilliant Corporation, TX, USA. The samples, Cals and QCs were added 0.1 ml NaOH (0.1 Molar) and further mixed for 10 min with 5 ml toluene in 1% amyl alcohol for 10 min. The samples were centrifuged at 1500g before the organic top layer volume was transferred and evaporated to dryness at 40 ºC before derivatization of OH-midazolam and OH-midazolam-d4 with 50 µl N-(tert-butyldimethylsilyl)-n-methyltrifluoroacetamide, with 1% tert-butyldimethylchlorosilane (TBDMSTFA from Sigma Aldrich, Milwaukee, WI, US) for 30 min at 60 ºC. Samples were evaporated to dryness and reconstituted in 50 µl ethyl acetate. The reconstituted samples (2 µl) were injected to and analytes separated on a HP-5MS, 30 m column (ID 0.25 mm, x-linked Ph Me Silicone from Agilent Technologies Palo Alto, CA, USA). The midazolam were quantified by the mass 310 (qualifier ion 325) and the OH-midazolam by the mass 389 (qualifier ion 400). Calibration ranges were 0.25-1500 and 0.25-250 ng/ml for midazolam and OH-midazolam, respectively. The r² were >0.999 for all calibration curves. The concentration of QCs (QC 1-3) was approx 0.05-0.3, 50 and 75% of the highest Cal standard for each analyte. LOQ (CV<20%, n=18) was 0.25 ng/ml for both midazolam and OH-midazolam. In the pre-run validation, CV (n=18x3) for QCs 1-3 was < 14.0 and 12.0% for midazolam and OH-midazolam, respectively. Inaccuracy (n=18x3) for QCs 1-3 was < 12.7 and 7.4% for midazolam and OH-midazolam, respectively. In-run, CV (n=14x3) for QCs 1-3 was < 6.0 and 14.6% for midazolam and OH-midazolam, respectively. Inaccuracy (n=14x3) for QCs 1-3 was < 10.5 and 9.3% for midazolam and OH-midazolam, respectively.
Urine concentrations of midazolam and its active metabolite OH-midazolam were determined essentially as serum concentrations. Patient samples, Cals and QCs (1.0 ml urine) were all spiked with d-4 deuterated midazolam and OH-midazolam as IS. Patient samples, Cals and QCs were prepared and analyzed as for serum (see above). Calibration ranges were 1.5-750 and 0.5-250 ng/ml for midazolam and OH-midazolam, respectively. The \( r^2 \) were >0.999 for all calibration curves. The concentration of QCs (QC 1-3) was 1.8, 50, and 75% of the highest Cal standard for both analytes. LOQ (CV<20%, n=18) was 1.5 and 0.5 ng/ml for midazolam and \( \alpha \)-OHM, respectively. In the pre-run validation, CV (n=18x3) for QCs 1-3 was < 5.9 and 6.9% for midazolam and OH-midazolam, respectively. Inaccuracy (n=18x3) for QCs 1-3 was < 8.5 and 5.7% for midazolam and OH-midazolam, respectively. In-run, CV (n=10x3) for QCs 1-3 was < 4.4 and 6.7% for midazolam and OH-midazolam, respectively. Inaccuracy (n=10x3) for QCs 1-3 was < 8.8 and 9.9% for midazolam and OH-midazolam, respectively.

Serum concentrations of fentanyl were analyzed by LCMSMS using the same analytical equipment as for morphine. Patient samples, Cals and QCs (500 µl serum or plasma) were all spiked with fentanyl-d5 as IS. All standard material was from Cerillant Corporation (Round Rock, TX, USA). The samples, Cals and QCs were mixed with 1 ml NH4 HCO3 (10mM, pH 9.3) and transferred to a solid phase column (SPE, C18, 200 mg, 3 ml Bond Elute from Varian Inc., Lake Forest, CA, USA). The analyte was eluted by methanol and the elution volume evaporated to dryness before reconstituted in 50 ml MP consisting of 65% methanol in 0.1% formic acid. The analytes in MP (5 µl) were injected to and separated on an Eclipse XDB-C8 column (5 µm, 150 mm × 4.6 internal diameter...
from Agilent Technologies Palo Alto, CA, USA). Fentanyl was quantified by the masses of 337/188. The calibration range was 0.02 -15.0 ng/ml. The concentration of QCs (QC 1-3) was 0.3, 50, and 75% of the highest Cal standard. The r² was >0.998 for all calibration curves. LOQ (CV<20%, n=18) was 0.02 ng/ml. In pre-run validation CV (n=18x3) for QCs 1-3 was < 4.9%, and inaccuracy was < 7.3%. In-run, CV (n=18x3) for QCs 1-3 was < 6.8% and inaccuracy < 10.3%.

Serum concentrations of propofol were analyzed essentially according to Bauer et al. using the same analytical equipment as for midazolam. (Bauer et al., 2004). Patient samples, Cals and QCs (300 µl serum or plasma) were all spiked with propofol-d18 as IS (CDN Isotopes Inc., Quebec, Canada). Calibration standard material was from Sigma Aldrich (Milwaukee, WI, US). The samples, Cals and QCs were mixed with 0.3 ml Toluene for 10 min, centrifuged at 10 000g, and 100 µl of toluene phase was transferred to auto-sampler vials. 2 µl was injected to and separated on a HP-5MS, 30 m column (ID 0.25 mm, x-linked Ph Me Silicone from Agilent Technologies Palo Alto, CA, USA). Propofol was quantified by the mass 163 (qualifier ion 117). The calibration range was 5.0-5000 ng/ml. The r² was >0.998 for all calibration curves. The concentration of QCs (QC 1-3) was 0.3, 50, and 75% of the highest Cal standard. LOQ (CV<20%, n=18) was 5.0 ng/ml. In the pre-run validation (n=18x3), CV for QCs 1-3 was < 6.9%, and inaccuracy was < 8.5%. In-run, CV (n=11x3) for QCs 1-3 was <9.8%, and inaccuracy was < 10.1%.

Pharmacokinetics
DMD #45567

Serum concentration data of morphine and midazolam were analyzed by non-compartmental techniques. The elimination rate constant ($\lambda_z$) was determined in Pharsight Win-Nonlin Professional 5.21 (Pharsight Corporation, USA) by manual curve fitting where points defining the log-linear portion of the elimination curve in each individual were selected by mutual agreement between two authors (OD and TWB). Elimination phase half-life ($T_{1/2}$) was defined as the natural logarithm of 2 divided by $\lambda_z$. Area under curve for the sampling period ($AUC_{\text{sample}}$) of morphine, midazolam, M3G, M6G, and OH-midazolam were calculated by Win-Nonlin using the linear trapezoidal method with linear interpolation. Assuming steady state conditions at the time of stopping morphine and midazolam infusions, serum concentrations at the time of discontinuation of infusions were defined as steady state concentrations ($C_{SS}$). Total clearance ($Cl_{tot}$) was calculated by dividing average infusion rate by $C_{SS}$. Apparent volume of distribution ($V$) was calculated by dividing $Cl_{tot}$ by $\lambda_z$. Renal elimination clearances ($Cl_R$) of morphine, M3G, and M6G, midazolam and OH-midazolam were calculated as amount excreted in urine during sampling (product of urine analyte concentration and volume of urine) divided by serum $AUC_{\text{sample}}$ of each substance (Krishna and Klotz, 1990).

For fentanyl and propofol, steady state was defined as two or more consecutive samples with a deviation of 10% or less during stable continuous infusion towards the end of the sampling period (Gibaldi and Perrier, 1982; Katz and Kelly, 1993). $C_{SS}$ was defined as the average steady state serum concentration. For each hour of infusion, the sum of drug infused and any boluses was combined to obtain the average hourly infusion rate. During
steady state of fentanyl and propofol, the respective Cl_{tot} were calculated by dividing the steady state infusion rate by C_{SS}.

Statistics
For sample size estimation, cardiac arrest patients were assumed to have a elimination half-life of 120 minutes with a standard deviation (SD) of 40 minutes for morphine (Bansinath et al., 1988; Berkenstadt et al., 1999). An inter-group difference of 30% was considered clinically interesting. Assuming hypothermia increases T_{1/2}, employing a significance level of 0.05, and a power of 0.80, the size for each group was 14 patients. On that basis, the aim was to include 15 patients in each group.

Descriptive data are reported as mean (SD) or median [semi-interquartile range (s-iqr)] as appropriate. Two-sided p-values ≤ 0.05 were considered significant. The 95% CI for inter group differences for the outcomes were also reported. Student's T-test was used for group comparisons where quantile-quantile plots indicated a normal distribution. Non-normally distributed data were compared with the Mann Whitney U test. Fisher’s exact test was used on categorical data. All statistical calculations were performed using R 2.15.0 statistical software package by the R Development Core Team, and the package exactRankTests (R Development Core Team, 2008).
Results

Fifteen of 25 screened patients treated with TH following cardiac arrest at St Olav’s University Hospital from September 2006 to November 2007 were included in the hypothermic group. Patients were excluded due to a history of substance abuse (n=3), renal failure (n=1), initial sedation with fentanyl/propofol (n=1), severe cardiovascular instability (n=1), because next of kin declined inclusion (n=2), or due to lack of available study personnel (n=2). Eight normothermic patients were matched and included from September 2006 to August 2009 following daily screening. Data from one hypothermic patient was excluded because equipment failure led to uncontrolled rewarming during the sampling period. Thus, 14 hypothermic and 8 normothermic patients remained for analysis (Fig. 2). Regarding fentanyl, three patients in the hypothermic group did not fulfill the criteria for steady state. For these three, the last observed serum concentration was used to calculate total clearance.

Patient characteristics

The groups were similar with respect to age, sex, height, and weight. Body mass index (BMI) was lower in the hypothermic compared to the normothermic group (p=0.04, Table 1). The groups were similar with respect to total bilirubin, plasma albumin, PT-INR, and SAPS II scores without GCS at admission. The hypothermic group had higher SAPS II scores without GCS the first 24 hours after admission and lower creatinine clearance (Table 2).
The groups were also similar with respect to drug amounts given and duration of infusions (mean (SD): Morphine 159 (56) vs. 141 (98) mg (p=0.65), midazolam 189 (83) vs. 171 (115) mg (p=0.70) and 15 (4.4) vs. 17 (3.9) hours (p=0.35). Median [min, max] core body temperature during the blood sampling period was 33.3 [32.4, 34.8]°C and 37.6 [36.2, 38.3]°C in the hypothermic and normothermic groups, respectively. At T₀, the groups were similar with respect to heart rate and diastolic blood pressure. However, systolic blood pressure and MAP was significantly lower in the hypothermic compared to the normothermic group, mean (SD) systolic blood pressure was 101 (10) vs. 122 (11) mmHg, and median [s-iqr] MAP was 72 [3.8] vs. 77 [3.8] mmHg, p=0.002 and 0.04, respectively. Core temperature was below 34°C for 2 hours before T₀ in one patient, 4 h in one patient and 7-17 h in the remaining patients. MAAS-scores were similar during sample collection, median [min, max] 0 [0, 0] in the hypothermic and 0 [1, 0] in the normothermic group, respectively. Calculated creatinine clearance in the hypothermic group was roughly half that of the normothermic group (median 66 vs. 137 ml/min, respectively, p<0.001).

Raw data are displayed as individual time concentration profiles of morphine, midazolam, and metabolites in semi-logarithmic plots in Figs. 3 and 4. A significant interindividual variation was observed, not least for midazolam. The elimination rate constants of M3G and M6G appeared smaller than those of morphine, indicating elimination rate limited kinetics for these metabolites in both groups. PK variables of morphine and midazolam are summarized in Table 3. The primary endpoint morphine T₁/₂ was significantly higher in the hypothermic patients (median[s-iqr] 266 [43] vs. 168
[11] minutes, p<0.01). Morphine Cl_{tot} and Cl_{R} were lower in the hypothermia group, while V was similar in both groups. For M3G and M6G, Cl_{R} appeared lower in the hypothermia group, but the differences were not statistically significant (Table 4). The C_{SS} ratios of M3G:morphine, M6G:morphine, and M3G:M6G were similar in both groups.

Midazolam T_{1/2}, Cl_{tot}, and V of midazolam did not differ between groups. The C_{SS} OH-midazolam:midazolam ratio was similar between groups (0.12 [0.03] vs. 0.13 [0.03]) (Table 3). Cl_{R} of midazolam and OH-midazolam was also similar in both groups.

Raw data are displayed as individual time concentration profiles of fentanyl and propofol with linear axes in Fig. 5. As for morphine and midazolam, interindividual variation was large.

Fentanyl Cl_{tot} (Table 4) was lower in the hypothermia group (726 [230] vs 1331 [678] ml/min, p<0.035). Propofol Cl_{tot} was lower in the hypothermic group (median [s-iqr] 2046 [305] vs. 2665 [223] ml/min, p=0.035) (Table 4).
Discussion

The major findings in this clinical study were that patients treated with therapeutic hypothermia, compared to the matched normothermic controls, showed significantly higher T\textsubscript{1/2} of morphine due to a lower clearance. The disposition of midazolam did not change significantly in hypothermic patients. Total clearances of fentanyl and propofol were lower in the TH group. Overall, PK variables showed large interindividual variation for all drugs.

The higher T\textsubscript{1/2} of morphine in the hypothermic patients was due to reduced clearance, as volume of distribution did not differ between the groups. The reduced clearance of morphine and subsequently an increased exposure is likely valid for several reasons. First, morphine PKs in the control group were similar to those of patients receiving general anesthesia, although PK data may differ substantially in ICU populations (Berkenstadt \textit{et al.}, 1999). Second, the difference between the groups for the primary outcome measure was statistically significant even if the study groups were smaller than planned. Third, our results complies with a previous study that reported 23% lower clearance and increased serum concentrations of morphine in TH treated neonates at approximately 33-34°C (Róka \textit{et al.}, 2008). However, in a study of dogs increased exposure to morphine during hypothermia (30°C) was caused by a combination of approximately 70% reduced clearance and a reduced volume of distribution, the latter in contrast to our findings (Bansinath \textit{et al.}, 1988).
The major elimination route of morphine is glucuronidation by uridine diphosphate glucuronosyltransferase 2B7 (UGT2B7) to M3G and M6G (Court et al., 2003). As enzyme activity usually decrease during hypothermia, the reduced clearance of morphine was likely caused by reduced activity of the glucuronidation enzymes. Reduced glucuronidation during hypothermia was previously demonstrated in isolated rabbit livers (Rink et al., 1956). In human neonates, hypothermia (33-34°C) increased serum concentrations by 40% and reduced clearance by 23% (Rink et al., 1956; Róka et al., 2008). A lower formation of metabolites during TH was expected to increase $C_{SS}$ of morphine and decrease $C_{SS}$ of metabolites, thus reducing the $C_{SS}$-ratios of the major metabolites M3G and M6G to morphine. Surprisingly, the $C_{SS}$-ratios did not differ, as the $C_{SS}$ of M3G and M6G were also higher. However, two factors may have affected these ratios. First, in both groups, elimination rate limited kinetics was observed for M3G and M6G. Reduced renal function is known to increase morphine glucuronide to morphine ratios (Faura et al., 1998). Second, the 50% reduction of creatinine clearance, a strong predictor of the renal clearances of morphine metabolites (Milne et al., 1992), suggests a reduction in renal excretion of M3G and M6G resulting in accumulation of these metabolites in serum. However, the calculated renal clearances of morphine metabolites only demonstrated a strong but not statistically significant trend towards reduction during hypothermia. This may represent a type II error, thus the effect of hypothermia on the renal clearance of morphine metabolites in man remains unknown.

The PKs of midazolam in the control group were similar to that described previously in critically ill patients (Løvstad et al., 1996). The observation that the half-life of
midazolam, in contrast to morphine; is not influenced by TH in these patients is based upon serial sampling, while the non-statistically significant trends toward lower clearance was based on one sample only. However, previous in vitro studies have shown reduction of CYP3A4 activity to $69\pm1\%$ at $32^\circ C$ (Fritz et al., 2005). Moreover, a systematic review that included both in vitro, animal, and human studies, concluded that CYP P450 activity is reduced by hypothermia at a rate of 7 to 22% per degree Celsius below $37^\circ$ (Tortorici et al., 2007). In previous studies in man, the effect of hypothermia on the clearance of midazolam vary from no statistically significant change (at $35.4^\circ C$, Hostler et al., 2010, although clearance was estimated to decrease 11.1% for each degree reduction of core temperature) to more than 100-fold ($<35^\circ C$, Fukuoka et al., 2004) compared to normothermia. Notably, volunteer studies may not be relevant for critically ill patients, and the design of the study of Fukuoka was quite different from the present study. On the other hand, the in vitro, maximum velocity of enzymatic metabolism of midazolam was reduced by approximately 13% at $33^\circ C$, (Empey et al., 2011). All over, current evidence is not sufficient to draw conclusions regarding the effect of TH under clinical conditions on the CYP3A4 mediated elimination of midazolam.

Despite the lack of statistically significant difference clearance for midazolam between the groups, clearance of the other CYP3A4 substrate fentanyl was lower in the hypothermic patients. This is supported by the current literature. In vitro, the maximum velocity of enzymatic metabolism of fentanyl was reduced by approximately 16% at $33^\circ C$ (Empey et al., 2011). In vivo, fentanyl pharmacokinetics in rats ($32.9\pm0.3^\circ C$), pigs ($31.6\pm0.2^\circ C$), and children, showed 20% reduced clearance, significantly reduced volume
of distribution, and essentially no elimination during profound hypothermia (18-25°C), respectively (Empey et al., 2011; Fritz et al., 2005; Koren et al., 1987).

For propofol, Cl\text{tot} was lower in hypothermic patients. This in accordance with a previous study during deep hypothermia (25-27°C) for cardiopulmonary bypass for heart surgery (Russell et al., 1989). However, a study in healthy volunteers (34±0°C) showed reduced inter-compartmental clearances, but not reduced total clearance, during TH (Leslie et al., 1995). Fentanyl, propofol, and morphine are drugs with high hepatic extraction ratios. Thus, the lower Cl\text{tot} of these drugs in the hypothermic patients may be also be due to the commonly recognized reduction in liver flow induced by hypothermia (Van den Broek et al., 2010). This would have explained the contrasting data in this study of the two CYP3A4 substrates midazolam and fentanyl. In a case series, morphine clearance was 53% lower in patients in septic shock (Macnab et al., 1986). However, liver blood flow was not investigated, and for fentanyl previous studies have questioned the dependency of clearance on liver flow (Olkkola et al., 1999).

The major advantage of this study was that it was conducted in patients under normal clinical care. However, this induces some limitations. First, despite the use of matching procedures and similar severity of disease between groups as assessed by SAPS II scores, the cases and controls were separable categories of patients within the ICU, and unknown confounders may occur. Second, although matching procedures were adequate, BMI was higher in the normothermic patients. However, obesity has little or no clinically significant effect on morphine dosing (Burns et al., 1989; Graves et al., 1983; Macintyre and Jarvis, 1996). Obesity increases volume of distribution and elimination half-life of
midazolam, and may thus obscure group differences (Greenblatt et al., 1984).

Polypharmacy is unavoidable in these patients and may cause unknown interactions. Atracurium and dopamine were only used in the hypothermic group. However, the literature does not support PK interactions between atracurium or dopamine and study drugs. Theoretically, dopamine may influence the clearance of high extraction drugs by changing liver flow, but it is unlikely that any such effect induced differences between groups because dopamine was used to maintain cardiac output. Third, only one $C_{SS}$ measurement was used to calculate $Cl_{tot}$ of morphine and midazolam in each patient. On the other hand, the experimental basis for the half-life determinations is strong. Fourth, because clearance will be overestimated if calculated prior to steady state, any difference between the groups would be reduced and not increased. Thus, the difference in $Cl_{tot}$ of fentanyl is a conservative estimate. Fifth, the sample size was lower than planned, especially for the control group. With large interindividual PK variations, the risk of making type 2 errors for secondary outcomes is large. Therefore, we also reported the 95% CI for inter group differences for the outcomes.

The findings of a reduced metabolism of morphine, fentanyl, and probably propofol during TH have clinical implications. Reduced metabolism can result in a relative overdose of these drugs if patients receive standard doses during TH. Due to difficulties in assessing sedation levels, not least in patients receiving neuromuscular blocking agents, too deep sedation is not easily recognized clinically. Thus, TH treated patients risk a too deep sedation. This may induce cardiovascular depression, and prolong the
time to recovery from sedation and analgesia. The latter may prolong mechanical ventilation and time to a valid assessment of cerebral function.

In conclusion, the elimination half-life of morphine was significantly higher due to reduced clearance in the hypothermic patients. The clearance of fentanyl and propofol were also lower in the hypothermic patients, indicating their infusion rates should be reduced during TH. No profound effect of TH was observed for the disposition of midazolam. Dose titration during TH is encouraged.
Acknowledgments

To helpful nurses and doctors at the ICU and Coronary Care Unit, St. Olav’s University Hospital for notification of patients and help with study procedures. Trine Andreassen for analysis of morphine and metabolites. Professor Klaus Olkkola for valuable feedback on the PK analysis and paper.

Authorship contributions

Participated in research design: Bjelland TW, Dale O, Haugen BO, Klepstad P, Nilsen T
Conducted experiments: Bjelland TW, Haugen BO, Klepstad P
Contributed analytic tools: Nilsen T
Performed data analysis: Bjelland TW, Dale O
Wrote or contributed to the writing of the manuscript: Bjelland TW, Dale O, Haugen BO, Klepstad P, Nilsen T
DMD #45567

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Court MH, Krishnaswamy S, Hao Q, Duan SX, Patten CJ, Von Moltke LL, and Greenblatt DJ (2003) Evaluation of 3'-azido-3'-deoxythymidine, morphine, and codeine as probe substrates for UDP-glucuronosyltransferase 2B7 (UGT2B7) in


Peberdy MA, Callaway CW, Neumar RW, Geocadin RG, Zimmerman JL, Donnino M, Gabrielli A, Silvers SM, Zaritsky AL, Merchant R, Vanden Hoek TL, and


Footnotes

Conflicts of interest: None

Footnotes:

a) This work was funded by the Norwegian University of Science and Technology, Trondheim, Norway.

b) Parts of the morphine and midazolam data were presented at Annual congress of the European Society of Intensive Care Medicine (ESICM) Lisbon 2008, at the annual meeting of the Norwegian Society of Anaesthesiology 2008, and at the annual winter meeting of The Norwegian Society of Pharmacology and Toxicology 2009. An updated abstract was presented at the 31st congress of the Scandinavian Society of Anaesthesiology and Intensive Care Medicine, 2011, Bergen, Norway. This paper will be included in the PhD thesis Pharmacological aspects of therapeutic hypothermia (Bjelland TW).

c) Reprint requests may be sent to the corresponding author

d) This study was carried out in accordance with the Declaration of Helsinki
Legends for figures

Figure 1:
Figure title: Figure 1 – Overview of treatment and study procedures
Figure legend: $T_{CA}$ – time of cardiac arrest, $T_s$ – time of start of sedation, $T_h$ – hypothermia established, $T_0$ – time of switch, $T_n$ – time of normothermia

Figure 2:
Figure title: Figure 2 – Flow chart of patient inclusion

Figure 3:
Figure title: Figure 3 – Time course of morphine, M3G, and M6G serum concentrations. Fat lines represent group mean, thin lines individual patients. Note that y-axis is logarithmic, and that the labels and scales of axes are the same in horizontal and vertical directions. For each patient, obvious outliers were omitted in the estimation of $\lambda_z$ of morphine. However, all points are included in this figure.

Figure 4:
Figure title: Figure 4 – Time course of midazolam and OH-midazolam serum concentrations. Fat lines represent group mean, thin lines individual patients. Note that y-axis is logarithmic, and that the labels and scales of axes are the same in horizontal and
vertical directions. For each patient, obvious outliers were omitted in the estimation of $\lambda_z$ of midazolam. However, all points are included in this figure.

Figure 5:

Figure title: Figure 5 – Time course of fentanyl and propofol serum concentrations. Fat lines represent group mean, thin lines individual patients. Note that y-axis is linear, and that the labels and scales of axes are the same in horizontal and vertical directions. Infusion rates varied both between and within individuals. All points are included in this figure to provide an unbiased presentation of the raw data.
### TABLE 1

**Patient characteristics**

Age, height, and weight evaluated with Student's t-test, BMI with Mann-Whitney U test, and sex with Fishers exact test. Values presented as mean (standard deviation), median [semi-interquartile range], or number of observations.

<table>
<thead>
<tr>
<th></th>
<th>Hypothermic</th>
<th>Normothermic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n=14</strong></td>
<td>n=8</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>60 (10)</td>
<td>65 (7)</td>
</tr>
<tr>
<td>Sex (males of total)</td>
<td>12 / 14</td>
<td>7 / 8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177 (11)</td>
<td>173 (9)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83 (18)</td>
<td>93 (16)</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>26 [1.2] *</td>
<td>31 [4.2]</td>
</tr>
</tbody>
</table>

*p<0.05"
### TABLE 2

**Study variables**

Student's t-test was used by default, non-normally distributed data variables were evaluated with Mann-Whitney U test. Values presented as mean (standard deviation), median [semi-interquartile range], median [min – max], or number of observations.

<table>
<thead>
<tr>
<th></th>
<th>Hypothermic</th>
<th>Normothermic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=14</td>
<td>n=8</td>
</tr>
<tr>
<td>SAPS II 0h without GCS</td>
<td>28 [1.5]</td>
<td>34 [3.8]</td>
</tr>
<tr>
<td>SAPS II 24h without GCS</td>
<td>42 (8.6)*</td>
<td>35 (5.7)</td>
</tr>
<tr>
<td>Plasma total bilirubin (µmol/L)</td>
<td>12 [5.4]</td>
<td>13 [0.5]</td>
</tr>
<tr>
<td>Plasma albumin (g/L)</td>
<td>31 [2.9]</td>
<td>31 [2.4]</td>
</tr>
<tr>
<td>PT-INR</td>
<td>1.2 [0.1]</td>
<td>1.2 [0.1]</td>
</tr>
<tr>
<td>Creatinine clearance during sampling (ml/minute)</td>
<td>66 [16]***</td>
<td>137 [21]</td>
</tr>
<tr>
<td>Morphin – infused amount before T₀ (mg)</td>
<td>159 (56)</td>
<td>141 (98)</td>
</tr>
<tr>
<td>Midazolam – infused amount before T₀ (mg)</td>
<td>189 (83)</td>
<td>171 (115)</td>
</tr>
<tr>
<td>Duration of infusion before T₀ (hours)</td>
<td>15 (4.4)</td>
<td>17 (3.9)</td>
</tr>
<tr>
<td>Hours hypothermic (&lt;34°C) before T₀</td>
<td>11 (4.6)</td>
<td>NA</td>
</tr>
<tr>
<td>Heart rate at T₀</td>
<td>62 [8]</td>
<td>56 [9]</td>
</tr>
<tr>
<td>Systolic blood pressure at T₀ (mmHg)</td>
<td>101 (10)**</td>
<td>122 (11)</td>
</tr>
<tr>
<td>Diastolic blood pressure at T₀ (mmHg)</td>
<td>59 (8)</td>
<td>58 (6)</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value 1</td>
<td>Value 2</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Mean arterial pressure at T₀ (mmHg)</td>
<td>72 [4]*</td>
<td>77 [4]</td>
</tr>
<tr>
<td>Duration of sampling period (hours)</td>
<td>8 [0]</td>
<td>8 [0]</td>
</tr>
<tr>
<td>Temperatures during sampling (°C)</td>
<td>33.3 [32.4 , 34.8]***</td>
<td>37.6 [36.2 , 38.3]</td>
</tr>
<tr>
<td>Fentanyl – infused amount during sampling (mg)</td>
<td>0.80 [0.16]</td>
<td>0.49 [0.26]</td>
</tr>
<tr>
<td>Propofol – Infused amount during sampling (mg)</td>
<td>1013 [322]</td>
<td>1344 [240]</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. T₀ - time of intervention (switch of sedatives). NA – not applicable. Creatinine clearance was calculated by dividing amount excreted (concentration * volume) by mean s-creatinine the day of inclusion and the day after.
TABLE 3

Pharmacokinetics of morphine and midazolam following discontinuation of continuous infusions

Student's t-test was used by default, non-normally distributed data variables were evaluated with Mann-Whitney U test. Values presented as mean (standard deviation), median [semi-interquartile range], or number of observations. Lower and upper bounds of confidence intervals presented as (lower, upper).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypothermic</th>
<th>Normothermic</th>
<th>95% CI for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=14</td>
<td>n=8</td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elimination half-life (min)</td>
<td>266 [43]**</td>
<td>168 [11]</td>
<td>(28, 123)</td>
</tr>
<tr>
<td>$\text{Cl}_\text{tot}$ (ml/min)</td>
<td>1201 [283]**</td>
<td>1687 [200]</td>
<td>(-1137, -139)</td>
</tr>
<tr>
<td>$V$ (L)</td>
<td>413 [89]</td>
<td>435 [28]</td>
<td>(-121, 140)</td>
</tr>
<tr>
<td>$\text{Cl}_R$ (ml/min)</td>
<td>66 [26]**</td>
<td>167 [26]</td>
<td>(-141, -56)</td>
</tr>
<tr>
<td>M3G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{Cl}_R$ (ml/min)</td>
<td>60 [19]</td>
<td>89 [20]</td>
<td>(-61, 3)</td>
</tr>
<tr>
<td>M6G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{Cl}_R$ (ml/min)</td>
<td>63 [23]</td>
<td>98 [13]</td>
<td>(-68, 1)</td>
</tr>
<tr>
<td>$C_{SS}$-ratios</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3G:morphine</td>
<td>7.6 (4.5)</td>
<td>7.6 (3.2)</td>
<td>(-3.4, 3.5)</td>
</tr>
<tr>
<td>M6G:morphine</td>
<td>1.3 (0.7)</td>
<td>1.4 (0.5)</td>
<td>(-0.6, 0.4)</td>
</tr>
<tr>
<td>M3G:M6G</td>
<td>5.6 [0.2]</td>
<td>5 [0.5]</td>
<td>(-0.3, 1.0)</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>------------------</td>
<td>-------------------------</td>
<td>---------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td><strong>Midazolam</strong></td>
<td><strong>Elimination half-life (min)</strong></td>
<td><strong>402 [104]</strong></td>
<td><strong>438 [86]</strong> ( -184 , 113 )</td>
</tr>
<tr>
<td></td>
<td><strong>Cl\textsubscript{tot} (ml/min)</strong></td>
<td><strong>383 [96]</strong></td>
<td><strong>517 [181]</strong> ( -421 , 74 )</td>
</tr>
<tr>
<td></td>
<td><strong>V (L)</strong></td>
<td><strong>200 [64]</strong></td>
<td><strong>324 [67]</strong> ( -224 , 21 )</td>
</tr>
<tr>
<td></td>
<td><strong>Cl\textsubscript{R} (ml/min)</strong></td>
<td><strong>0.97 [0.4]</strong></td>
<td><strong>0.55 [0.5]</strong> ( -1.7 , 0.5 )</td>
</tr>
<tr>
<td><strong>OH-midazolam</strong></td>
<td><strong>Cl\textsubscript{R} (ml/min)</strong></td>
<td><strong>1.3 [0.4]</strong></td>
<td><strong>2.4 [0.8]</strong> ( -1.7 , 0.5 )</td>
</tr>
<tr>
<td><strong>C\textsubscript{SS}-ratio</strong></td>
<td><strong>OH-mid:mid</strong></td>
<td><strong>0.12 [0.03]</strong></td>
<td><strong>0.13 [0.03]</strong> ( -0.05 , 0.06 )</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. C\textsubscript{SS} - steady state concentration at the end of morphine and midazolam infusions, Cl\textsubscript{R} renal clearance, Cl\textsubscript{tot} total clearance, V - apparent volume of distribution, M3G - morphine-3-glucuronide, M6G - morphine-6-glucuronide, mid – midazolam, OH-mid – OH-midazolam.
TABLE 4

Clearances of fentanyl and propofol during TH

Significance evaluated with Mann-Whitney U test. Values presented as median [semi-interquartile range]. Lower and upper bounds of confidence intervals presented as (lower, upper).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>Hypothermic</th>
<th>Normothermic</th>
<th>95% CI for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n=14</td>
<td>n=8</td>
<td></td>
</tr>
<tr>
<td>Fentanyl</td>
<td>ml/min</td>
<td>726 [230]*</td>
<td>1331 [678]</td>
<td>(-1440, -27)</td>
</tr>
<tr>
<td>Propofol</td>
<td>ml/min</td>
<td>2046 [305]*</td>
<td>2665 [223]</td>
<td>(-1343, -106)</td>
</tr>
</tbody>
</table>

*p<0.05. CI - confidence interval for inter group difference. Cl_{tot} - total clearance.
Figure 1

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Enrolment

Hypothermic group

Assessed for eligibility (n=25)

Excluded (n=10)
  - Not meeting inclusion criteria (n=6)
  - Next of kin denied participation (n=2)
  - Investigator unavailability (n=2)

Included (n=15)

Excluded from data analyses (n=1)
  - Uncontrolled rewarming during sampling period (n=1)

Lost to follow-up (n=0)
Discontinued intervention (n=0)

Analysed (n=14)

Normothermic group

Patients matching age, sex, duration of infusion and eligible for inclusion (n=8)

Excluded (n=0)
  - Next of kin denied participation (n=0)

Included (n=8)

Excluded from data analyses (n=0)

Lost to follow-up (n=0)
Discontinued intervention (n=0)

Analysed (n=8)

Figure 2
Figure 3
Figure 4

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Figure 5