Effects of Hypothermia on the Disposition of Morphine, Midazolam, Fentanyl, and Propofol in Intensive Care Unit Patients

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ABSTRACT

Therapeutic hypothermia (TH) may induce pharmacokinetic changes that may affect the level of sedation. We have compared the disposition of morphine, midazolam, fentanyl, and propofol in TH with normothermia in man. Fourteen patients treated with TH following cardiac arrest (33–34°C) were compared with eight 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. During TH, morphine elimination half-life (t1/2) was significantly higher, while total clearance (CLtot) was significantly lower (median [semi-interquartile range (s-iqr)]: t1/2, 266 (43) versus 168 (11) minutes, P < 0.01; CLtot, 1201 (283) versus 1687 (200) ml/min, P < 0.01. No significant differences were seen for midazolam. CLtot of fentanyl and propofol was significantly lower in hypothermic patients [median (s-iqr)]: fentanyl, 726 (230) versus 1331 (678) ml/min, P < 0.05; propofol, 2046 (305) versus 2665 (223) ml/min, P < 0.05. Compared with the matched, normothermic intensive care unit patients, t1/2 of morphine was significantly higher during TH. CLtot was lower during TH for morphine, fentanyl, and propofol but not for midazolam. Reducing the infusion rates of morphine, fentanyl, and propofol during TH 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 12–24 hours) (Bernard et al., 2002; Hypothermia after Cardiac Arrest Study Group, 2002; Peberdy et al., 2010). Patients treated with TH are given sedatives and analgesics 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 physiologic changes that affect drug disposition and action through changes in both metabolism and distribution (Kadar et al., 1982; Koren et al., 1987; Bansinath et al., 1988; Alcaraz et al., 1989; Beaufort et al., 1995; Leslie et al., 1995; Asokumar et al., 1998; Caldwell et al., 2000; Fukuoaka et al., 2004; Tortorici et al., 2007; Arpino and Greer, 2008; Polderman, 2009). Reduced metabolism due to changes in enzyme activity during hypothermia may increase drug serum levels, and thus drug effects and duration of action (Polderman, 2004; Arpino and Greer, 2008). However, the effects of hypothermia on the activity of different enzymes vary; whereas 10°C lower temperature reduced cytochrome P450 (P450) activity on diazepam by 22%, conjugation of oxazepam was reduced by only 14% (Mortensen and Dale, 1995). In addition, whereas CYP3A and CYP2E activity as measured by clearance of midazolam and chlorozoxazon was approximately 50 and 60% lower in rats given cardiac arrest and TH compared with the control group, no differences were demonstrated for CYP2C/D (Zhou et al., 2011). This substrate specificity implies that the effects of hypothermia on pharmacokinetics (PK) may vary between drugs (Mortensen and Dale, 1995; Zhou et al., 2011; Zhou and Poloyac, 2011).

Morphine is metabolized by uridine diphosphate glucuronosyltransferase 2B7 (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 with
normothermic controls (Rink et al., 1956; Bansinath et al., 1988; Róka et al., 2008). CYP3A4 metabolizes both midazolam and fentanyl. A systematic review found that 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 activity of midazolam was reduced by approximately 13% (Fritz et al., 2005; Empey et al., 2012). 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 (Russell et al., 1989; Leslie et al., 1995).

In summary, reduced elimination during hypothermia has been shown for morphine, midazolam, and fentanyl in both animals and man (Koren et al., 1987; Bansinath et al., 1988; Fukuoka et al., 2004; Fritz et al., 2005). Study results are less uniform regarding apparent volume of distribution, where it was reported increased for midazolam and reduced for morphine and fentanyl (Koren et al., 1987; Bansinath et al., 1988; Fukuoka et al., 2004). However, these studies were performed in vitro, in animals, children, and healthy volunteers, which are settings that limit their validity with regard to adult patients treated with TH after cardiac arrest. However, the existing evidence suggests that hypothermia can induce clinically significant PK changes that increase the risk of detrimental oversedation; thus more knowledge on drug disposition during TH is needed (Koren et al., 1987; Polderman, 2004; Pedersen et al., 2007; Tortorici et al., 2007; Bjelland et al., 2010). 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 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 cardiopulmonary resuscitation. 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 to the study drugs were excluded. Controls were normothermic (36–38°C) intensive care patients matched for 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 MTRÆ Advanced Technologies Ltd., Rehovot, Israel) 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 mm Hg. and hypotension was treated with crystalloid fluids and vasopressor agents (norepinephrine, dopamine). Target diuresis was 1 ml/kg/h. 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, Asker, Norway) 5 mg/ml and midazolam (Alpharma AS, Oslo, Norway, or F. Hoffmann-La Roche AG, Basel, Switzerland) 5 mg/ml. ICU nurses and the attending physician titrated doses to maintain a Motor Activity Assessment Scale (MAAS) score of 0–1 and to avoid shivering (Devlin et al., 1999). During infusions of neuromuscular blocking agents, sedatives and analgesics were given at 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 hours before T0. Patient core temperature was below 34°C for at least 2 hours before T0. At T0, the infusions of morphine and midazolam were stopped and replaced with infusions of fentanyl 0.05 mg/ml (Alpharma AS) and propofol 10 mg/ml (Alpharma AS). Fentanyl was administered at 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 hypothymic group. To compare severity, SAPS II scores were therefore calculated without GCS, but otherwise as reported by Le Gall et al. (1993). Current medications and infusation rates of study drugs were retrieved from the medical records and infusion pumps. Total serum concentrations of bilirubin, albumin, and creatinine and prothrombin time–international normalized ratio were analyzed using standard clinical chemistry methods. MAAS scores, intra-arterial blood pressure, heart rate, urine production, and rectal temperature were recorded at each blood sampling point. Finally, the amount of creatinine excreted during the collection interval (concentration × volume) was divided by serum albumin 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 Tₚ, in Vacutainer tubes (Greiner Bio One, Kremsmünster, 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 at 1,500g before the organic top-layer volume was transferred and evaporated to dryness at 40°C before derivatization of OH-midazolam and OH-midazolam-d₄ with 50 μl N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (TBDMSTFA, Sigma-Aldrich, Milwaukee, WI) for 30 minutes at 60°C. Samples were evaporated to dryness and reconstituted in 50 μl ethyl acetate. The reconstituted samples (2 μl) were injected to and analyzed separated on an HP-5MS column (crosslinked 5 % PH ME Silicone, 30m × 0.25mm × 0.25μm Film Thickness; Agilent Technologies, Palo Alto, CA). The midazolam was quantified by the mass 310 (qualifier ion 325) and the OH-midazolam by the mass 389 (qualifier ion 400). Calibration ranges were 0.25–150 and 0.25–250 ng/ml for midazolam and OH-midazolam, respectively. The r²s were >0.999 for all calibration curves. The concentration of QCs (QC 1–3) was approximately 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 prerun validation, CV (n = 18 × 3) for QCs 1–3 was <14.0% and 12.0% for midazolam and OH-midazolam, respectively. Inaccuracy (n = 18 × 3) for QCs 1–3 was <12.7 and 7.4% for midazolam and OH-midazolam, respectively. In-run, CV (n = 14 × 3) for QCs 1–3 was <6.0 and 14.6% for midazolam and OH-midazolam, respectively. Inaccuracy (n = 14 × 3) 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 μl urine) were all spiked with d₄ deuterated midazolam and OH-midazolam-d₄ as IS. All standard material was from Cerilliant Corporation (Round Rock, TX). The samples, Cals, and QCs were added to 0.1 ml NaOH (0.1 M) and further mixed for 10 minutes with 5 ml tolune in 1% amyl alcohol for 10 minutes. The samples were centrifuged at 1,500g before the organic top-layer volume was transferred and evaporated to dryness at 40°C before derivatization of OH-midazolam and OH-midazolam-d₄ with 50 μl N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (TBDMSTFA, Sigma-Aldrich, Milwaukee, WI) for 30 minutes at 60°C. Samples were evaporated to dryness and reconstituted in 50 μl ethyl acetate. The reconstituted samples (2 μl) were injected to and analyzed separated on an HP-5MS column (crosslinked 5 % PH ME Silicone, 30m × 0.25mm × 0.25μm Film Thickness; Agilent Technologies, Palo Alto, CA). The midazolam was 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 0.25–2500 ng/ml for midazolam and OH-midazolam, respectively. The r²s were >0.999 for all calibration curves. The concentration of QCs (QC 1–3) was approximately 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 prerun validation, CV (n = 18 × 3) for QCs 1–3 was <14.0% and 12.0% for midazolam and OH-midazolam, respectively. Inaccuracy (n = 18 × 3) for QCs 1–3 was <12.7 and 7.4% for midazolam and OH-midazolam, respectively. In-run, CV (n = 14 × 3) for QCs 1–3 was <6.0 and 14.6% for midazolam and OH-midazolam, respectively. Inaccuracy (n = 14 × 3) for QCs 1–3 was <10.5 and 9.3% for midazolam and OH-midazolam, respectively.

In the hyperthermic group, all serum concentrations of midazolam and its active metabolite OH-midazolam as well as serum midazolam and OH-midazolam were determined essentially as serum concentrations. Patient samples, Cals, and QCs (1.0 μl urine) were all spiked with d₄ deuterated midazolam and OH-midazolam-d₄ as IS. All standard material was from Cerilliant Corporation (Round Rock, TX). The samples, Cals, and QCs were added to 0.1 ml NaOH (0.1 M) and further mixed for 10 minutes with 5 ml tolune in 1% amyl alcohol for 10 minutes. The samples were centrifuged at 1,500g before the organic top-layer volume was transferred and evaporated to dryness at 40°C before derivatization of OH-midazolam and OH-midazolam-d₄ with 50 μl N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (TBDMSTFA, Sigma-Aldrich, Milwaukee, WI) for 30 minutes at 60°C. Samples were evaporated to dryness and reconstituted in 50 μl ethyl acetate. The reconstituted samples (2 μl) were injected to and analyzed separated on an HP-5MS column (crosslinked 5 % PH ME Silicone, 30m × 0.25mm × 0.25μm Film Thickness; Agilent Technologies, Palo Alto, CA).
standard material was from Sigma-Aldrich. The samples, Cals, and QCs were mixed with 0.3 ml toluene for 10 minutes, centrifuged at 10,000g, and 100 μl of toluene phase was transferred to autosampler vials. Two microliters was injected to and separated on an Agilent HP-5MS 30-m column (see above). Propofol was quantified by the mass 163 (qualifier ion 117). The calibration range was 5.0–5000 ng/ml. The \( r^2 \) 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 (n = 18) was 5.0 ng/ml. In the prerun validation (n = 18 × 3), CV for QCs 1–3 was <6.9% and inaccuracy was <8.5%. In-run, CV (n = 11 × 3) for QCs 1–3 was <9.8% and inaccuracy was <10.1%.

**PK.** Serum concentration data for morphine and midazolam were analyzed by noncompartmental techniques. The elimination rate constant (\( \lambda_e \)) was determined in Pharsight Win-Nonlin Professional 5.21 (Pharsight Corporation, Sunnyvale, CA) 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 (O.D. and T.W.B.). Elimination-phase half-life (\( t_{1/2} \)) was defined as the natural logarithm of 2 divided by \( \lambda_e \). Areas under the 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 (CSS). Total clearance (CL\(_{\text{tot}}\)) was calculated by dividing average infusion rate by CSS. Apparent volume of distribution (V) was calculated by dividing CL\(_{\text{tot}}\) by \( \lambda_e \). Renal elimination clearances (CL\(_{\text{R}}\)) 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 areas under the curve for the sampling period 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 toward the end of the sampling period (Gibaldi and Perrier, 1982; Katz and Kelly, 1993). CSS 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\(_{\text{tot}}\) were calculated by dividing the steady-state infusion rate by CSS.

**Statistics.** For sample size estimation, cardiac arrest patients were assumed to have a \( t_{1/2} \) of 120 minutes with an S.D. of 40 minutes for morphine (Bansinath et al., 1988; Berkenstadt et al., 1999). An intergroup 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 (S.D.) or median [semi-interquartile range (s-iqr)] as appropriate. Two-sided \( P \) values ≤0.05 were considered significant. The 95% confidence interval for intergroup 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), or 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 were 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

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**Fig. 2.** Flowchart of patient inclusion.
TABLE 1
Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hypothermic (n =14)</th>
<th>Normothermic (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>60 (10)</td>
<td>65 (7)</td>
</tr>
<tr>
<td>Sex (males/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

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 was lower in the hypothermic compared with the normothermic group (P = 0.04) (Table 1). The groups were similar with respect to total bilirubin, plasma albumin, prothrombin time—international normalized ratio, 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 (S.D.): morphine dose, 159 (56) versus 141 (98) mg (P = 0.65); midazolam dose, 189 (83) versus 171 (115) mg (P = 0.70); and duration, 15 (4.4) versus 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 were significantly lower in the hypothermic group compared with the normothermic group (mean (S.D.) systolic blood pressure was 101 (10) versus 122 (11) mm Hg; and median (s-iqr) MAP was 72 (3.8) versus 77 (3.8) mm Hg (P = 0.002 and 0.04, respectively). Core temperature was below 34°C for 2 hours before T₀ in one patient, and 7–17 hours in the remaining patients. MAAS scores were similar during sample collection [median (min, max)]: 0 (0, 0) in the hypothermic group and 0 (1, 0) in the normothermic group. Calculated creatinine clearance in the hypothermic group was roughly half that of the normothermic group (median 66 versus 137 ml/min; P < 0.001).

Raw data are displayed as individual time-concentration profiles of morphine, midazolam, and metabolites in semilogarithmic 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 for 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) versus 168 (11) minutes (P < 0.01). Morphine CLtot and CR were lower in the hypothermia group, whereas V was similar in both groups. For M3G and M6G, CR appeared lower in the hypothermia group, but the differences were not statistically significant (Table 4). The C_SSS ratios of M3G/morphine, M6G/morphine, and M3G/M6G were similar in both groups.

Midazolam t₁/₂, CLtot, and V did not differ between groups. The OHmidazolam/midazolam C_SSS ratio was similar between groups [0.12 (0.03) versus 0.13 (0.03)] (Table 3). CLtot of midazolam and OHmidazolam 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 CLtot (Table 4) was lower in the hypothermic group [726 (230) versus 1331 (678) ml/min; P < 0.035]. Propofol CLtot was lower in the hypothermic group [median (s-iqr) 2046 (305) versus 2665 (223) ml/min; P = 0.035] (Table 4).

Discussion

The major findings in this clinical study were that patients treated with TH, compared with the matched normothermic controls, showed a significantly higher t₁/₂ of morphine due to a lower clearance. The

TABLE 2
Study variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypothermic (n = 14)</th>
<th>Normothermic (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAPS II 0 h without GCS</td>
<td>28 [1.5]</td>
<td>34 [3.8]</td>
</tr>
<tr>
<td>SAPS II 24 h without GCS</td>
<td>42 (8.6)*</td>
<td>35 (5.7)</td>
</tr>
<tr>
<td>Plasma total bilirubin (µM)</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/min)²</td>
<td>66 [16]**</td>
<td>137 [21]</td>
</tr>
<tr>
<td>Morphine, amount infused before T₀ (mg)</td>
<td>159 (56)</td>
<td>141 (98)</td>
</tr>
<tr>
<td>Midazolam, amount infused before T₀ (mg)</td>
<td>189 (83)</td>
<td>171 (115)</td>
</tr>
<tr>
<td>Duration of infusion before T₀ (h)</td>
<td>15 (4.4)</td>
<td>17 (3.9)</td>
</tr>
<tr>
<td>Heart rate at T₀ (bpm)</td>
<td>11 (4.6)</td>
<td>N.A.</td>
</tr>
<tr>
<td>Systolic blood pressure at T₀ (mm Hg)</td>
<td>62 [8]</td>
<td>56 [9]</td>
</tr>
<tr>
<td>Diastolic blood pressure at T₀ (mm Hg)</td>
<td>101 (10)**</td>
<td>122 (11)</td>
</tr>
<tr>
<td>Mean arterial pressure at T₀ (mm Hg)</td>
<td>59 (8)</td>
<td>58 (6)</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, amount infused during sampling (mg)</td>
<td>0.80 [0.16]</td>
<td>0.49 [0.26]</td>
</tr>
<tr>
<td>Propofol, amount infused during sampling (mg)</td>
<td>1013 [322]</td>
<td>1344 [240]</td>
</tr>
</tbody>
</table>

² Creatinine clearance was calculated by dividing amount excreted (concentration × volume) by mean creatinine the day of inclusion and the day after.

* P < 0.05; ** P < 0.01; *** P < 0.001.
disposition of midazolam did not change significantly in hypothermic patients. $\text{Cl}_{\text{tot}}$s of fentanyl and propofol were lower in the TH group. Overall, PK variables showed large interindividual variation for all drugs.

The higher $t_{1/2}$ 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 subsequent increased exposure is likely valid for several reasons. First, the PK of morphine in the control group were similar to those of patients receiving general anesthesia, although PK data may differ substantially in ICU populations (Berkenstadt 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 complied with a previous study that reported 23% lower

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**Fig. 3.** Time course of morphine, M3G, and M6G serum concentrations. Thick lines represent group mean, thin lines individual patients. Note that the $y$-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.
clearance and increased serum concentrations of morphine in TH-treated neonates at approximately 33–34°C (Róka 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 et al., 1988).

### TABLE 3

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

CLR, renal clearance; CL_{tot}, total clearance; C_{SS}, steady-state concentration at the end of morphine and midazolam infusions; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; V, apparent volume of distribution.

**P < 0.01; ***P < 0.001.
The major elimination route of morphine is glucuronidation by UGT2B7 to M3G and M6G (Court et al., 2003). As enzyme activity usually decreases 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 were observed for M3G and M6G. Reduced renal function is known to increase morphine glucuronide/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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hypothemic (n = 14) Group</th>
<th>Normothemic (n = 8) Group</th>
<th>95% CI for Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl</td>
<td>726 [230]*</td>
<td>1331 [678]</td>
<td>(-1400, -27)</td>
</tr>
<tr>
<td>Propofol</td>
<td>2046 [305]*</td>
<td>2665 [223]</td>
<td>(-1343, -106)</td>
</tr>
</tbody>
</table>

* P < 0.05.
calculated renal clearances of morphine metabolites only demonstrated a strong but not statistically significant trend toward reduction during hypothermia. This may represent a type 2 error; thus the effect of hypothermia on the renal clearance of morphine metabolites in man remains unknown.

The PK of midazolam in the control group were similar to that described previously in critically ill patients (Løwstad et al., 1996). The observation that the half-life of midazolam, in contrast to morphone, is not influenced by TH in these patients is based on serial sampling, whereas the statistically nonsignificant trend toward lower clearance was based on one sample only. However, previous in vitro studies have shown reduction of CYP3A4 activity to 69 ± 1% at 32°C (Fritz et al., 2005). Moreover, a systematic review that included in vitro, animal, and human studies concluded that P450 activity is reduced by hypothermia at a rate of 7 to 22% per degree Celsius below 37°C (Tortorici et al., 2007). In previous studies in man, the effects of hypothermia on the clearance of midazolam vary from no statistically significant change (at 35.4°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 (at <35°C [Fukuoka et al., 2004]) compared with 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°C (Empey et al., 2012). Overall, 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 differences in clearance of 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°C (Empey et al., 2012). In vivo, fentanyl PK in rats (32.9 ± 0.3°C), pigs (31.6 ± 0.2°C), and children showed 20% reduced clearance, significantly reduced volume of distribution, and essentially no elimination during profound hypothermia (18–25°C), respectively (Koren et al., 1987; Fritz et al., 2005; Empey et al., 2012).

For propofol, CLtot was lower in hypothermic patients. This is in accordance with a previous study during deep hypothermia (25–27°C) for cardiopulmonary bypass in heart surgery (Russell et al., 1989). However, a study in healthy volunteers (34 ± 0°C) showed reduced intercompartmental clearances, but not reduced CLtot, during TH (Leslie et al., 1995). Fentanyl, propofol, and morphine are drugs with high hepatic extraction ratios. Thus, the lower CLtot 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 for 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 (Olkola 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, body mass index was higher in the normothermic patients. However, obesity has little or no clinically significant effect on morphine dosing (Graves et al., 1983; Burns et al., 1989; Macintyre and Jarvis, 1996). Obesity increases the volume of distribution and t1/2 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 used only 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 CSS measurement was used to calculate CLtot of morphine and midazolam in each patient. On the other hand, the experimental basis for the t1/2 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 CLtot 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% confidence intervals for intergroup 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 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 t1/2 of morphine was significantly higher due to reduced clearance in the hypothermic patients. The clearances of fentanyl and propofol were also lower in the hypothermic patients, indicating that 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.

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Authorship Contributions

Participated in research design: Bjelland, Dale, Haugen, Klepstad, Nilsen.
Conducted experiments: Bjelland, Haugen, Klepstad.
Contributed new reagents or analytic tools: Nilsen.
Performed data analysis: Bjelland, Dale.
Wrote or contributed to the writing of the manuscript: Bjelland, Dale, Haugen, Klepstad, Nilsen.

References


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