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Detection of morphine-3-sulfate and morphine-6-sulfate in human urine and plasma, and formation in liver cytosol

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LC-MS/MS, morphine, morphine-3-sulfate, morphine-6-sulfate, plasma, urine

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Abstract

Morphine is still the mainstay in treatment of severe pain and is metabolized in the liver mainly by glucuronidation, partly to the pharmacologically active morphine-6-glucuronide (M6G). The sulfation pathway has attracted much less attention but may also form active metabolites. The aim of the present study was to study two sulfate metabolites of morphine in humans. Urine and plasma from newborns, adult heroin addicts, and terminal cancer patients was analyzed for the presence of morphine-3-sulfate (M3S) and morphine-6-sulfate (M6S) by a new liquid chromatography – tandem mass spectrometry (LC-MS/MS) method. In addition, morphine sulfation was studied in vitro in human liver cytosol preparations. M3S was present in urine and plasma from all study groups although at lower concentrations than morphine-3-glucuronide (M3G). The plasma M3S/M3G ratio was 30 times higher in newborns than in adults indicating that the relative sulfation is more important at early stage of life. M6S was measurable in only one plasma sample from a newborn patient, and in one of the urine sample from the drug testing group. The incubation of morphine with liver cytosol extracts resulted in approximately equal rate of formation of both M3S and M6S. In conclusion, sulfation of morphine is catalyzed in human liver but this minor metabolic pathway probably lacks clinical significance. The M6S metabolite is formed at a low rate, making it undetectable in most individuals.

Abbreviation

LC-MS/MS, liquid chromatography – tandem mass spectrometry; LLOQ, lowest limit of quantification; M3G, morphine-3-glucuronide; M3S, morphine-3-sulfate; M6G, morphine-6-glucuronide; M6S, morphine-6-sulfate; M-d_3, morphine-d_3; PAPS, 3′-phosphoadenosine 5′-phosphosulfate lithium salt hydrate; SULT, sulfotransferase.

Introduction

Morphine has been used as a drug by humans for thousands of years for its analgetic effects. It is metabolized in the liver mainly by three different pathways, glucuronidation, sulfation, and *N*-demethylation (Milne et al. 1996; Aderjan and Skopp 1998; Projean et al. 2003). The main metabolic pathway is glucuronidation leading to the inactive morphine-3-glucuronide (M3G) and the pharmacologically active morphine-6-glucuronide (M6G). The human kinetics of morphine glucuronides have been thoroughly investigated ever since bioanalytical methods for their determination in blood became available (Svensson et al. 1982; Milne et al. 1996). The sulfation pathway, on the other hand, has attracted much less attention. Although morphine-3-sulfate (M3S) is generally considered to be present in humans, the presence of morphine-6-sulfate (M6S) still remains to be demonstrated. The

enzymes responsible for formation of M3S and M6S are still not determined.

In general, sulfation is an important pathway for the intermediary metabolism during fetal life, whereas glucuronidation is more important in adults (Besunder et al. 1988).

To date, 12 human sulfotransferase (SULT) genes have been identified. The SULT-enzymes constitute the major detoxification defenses in fetuses and the expression of the SULT-isoforms varies during development. SULT1E1 is expressed during the earliest stages of human gestation, but later declines in favor of SULT2A1 which levels continue to increase during fetal development and the first year of life (Duanmu et al. 2006). According to in vitro data, SULT1A3 is the major enzyme for the sulfation of morphine in human hepatocytes (Kurogi et al. 2014).

The glucoronidation enzymes (UGT) are a family of enzymes of which UGT2B7 has been shown to be the major isoenzyme important for the formation of M3G and M6G (Chau et al. 2014).

In newborns, morphine has been shown to be conjugated both with sulfate and glucuronic acid, and the ratio of M3S/morphine was higher in urine of premature and full-term newborns than in older children suggesting that morphine sulfation decreases after the neonatal period (Choonara et al. 1990). No M6S was measurable in urine in that study. The low concentrations of M3S suggest that this is a minor metabolic pathway.

To what degree sulfation of morphine is of importance in terminal cancer patients is not known. However, morphine kinetics seems not to be altered in any significant way in high-dosed cancer patients (Sawe et al. 1981; Rane et al. 1982; Sawe et al. 1983b). It may be speculated, however, that the liver may lose some glucuronidation activity in favor of sulfation during the last period of life in which many physiological functions may change or wane (Long 1996).

Analysis of low concentrations of M3S in urine in pediatric patients was made using liquid chromatography with UV-detection and liquid chromatography – tandem mass spectrometry (LC-MS/MS) (Choonara et al. 1990; Andersson et al. 2012). Research on morphine sulfates has been hampered by the lack of commercially available reference material and sensitive methods (Andersson et al. 2012).

The pharmacological activity of M6G is considered to contribute to the effects of morphine (Portenoy et al. 1992). In addition, during chronic administration of morphine, M6G may accumulate and cause prolonged effects in patients with reduced kidney function (Rane et al. 1982; Sawe et al. 1983a,b). One reason of interest in morphine sulfate conjugates is that in earlier animal studies performing tail-flick tests, administration of M6S resulted in a 30-fold greater analgesic potency compared to administration of morphine. M6S may possess pharmacological activity similar to M6G. Regarding M3S, it has been described to have none or little analgesic potency. The sulfonate moiety at the 3′-position may also obstruct binding to the opioid receptors (Houdi et al. 1996; Crooks et al. 2006; Rook et al. 2006). Given this situation, it is important to study their possible accumulation during chronic treatment with morphine.

The aim of the present study was to identify and determine sulfate metabolites of morphine in newborns, adult heroin addicts, and terminal cancer patients. Urine and plasma samples were analyzed for the presence of M3S and M6S using a sensitive and selective LC-MS/MS method. In addition, the metabolic capability of morphine sulfation was studied in human liver cytosols in vitro.

Materials and Methods

Chemicals and reagents

The following substances were obtained from LGC Standards (Teddington, UK): morphine, M3G, M6G, morphine-d_3 (M-d_3), M3G-d_3, and M6G-d_3. M3S and M6S were prepared inhouse according to published procedure (Andersson et al. 2012). 3′-Phosphoadenosine 5′-phosphosulfate lithium salt hydrate (PAPS) was obtained from Sigma-Aldrich (St. Louis, MO). Formic acid (proanalysis quality) and acetonitrile (LiChrosolv isocratic grade for liquid chromatography) were from Merck KGaA (Darmstadt, Germany). Methanol (HiPerSolv CHROMANORM for HPLC gradient grade) was obtained from VWR International (Radnor, PA). Ultrapure water was produced inhouse by a Milli-Q Millipore Water system (EMD Millipore, Billerica, MA).

Cancer patients treated with morphine

Eleven cancer patients, six females and five males aged 30–81, treated with morphine in slow-release tablets (Dolcontin, Pfizer, New York, NY) or treated with morphine via continuous, subcutaneous infusion with a Cadd-Legacy pump were included in the study. The patients were recruited from ASIH Långbro Parks Palliative Care Unit, Stockholm, Sweden, during January–September 2013. Written informed consent was obtained from all study participants. The study was approved by the local Ethics Committee (Dnr: 2012/1839-31/4) and was performed in accordance with the declaration of Helsinki. From each patient, one urine and blood samples were collected at the same time point. The time between the last intake of dose and sample collection was recorded in the patients taking tablets. When possible, samples from the same

patients (urine and blood) were collected twice. The median survival time of the patients was 4 weeks (range 5 days to 1 year) after the blood samples were drawn.

Newborns treated with morphine

Twenty-one newborns treated with morphine by continuous infusion as part of pain treatment, for example, during surgical intervention, were recruited from Astrid Lindgrens Children's Hospital, Karolinska University Hospital, Stockholm, Sweden. Written informed consent was obtained from all patients and parents/guardians. The study was approved by the local Ethics Committee (Dnr: 2009/1251-31/4 and 2010/570-32) and was performed in accordance with the Declaration of Helsinki. From each patient a maximum of four blood samples were collected from an existing catheter. No more than 0.8 mL blood was collected each time.

Heroin drug addicts

Routine urine drug testing samples were used if there was a remaining surplus after analysis. The number of samples selected was 200 and the sample selection was made based on the presence of the heroin metabolite 6-acetylmorphine measured by LC-MS/MS (Andersson et al. 2013). This procedure was approved by the local Ethics Committee (Dnr: 2008/1087-32).

In vitro study

Human fetal liver tissue was obtained from a biobank as described previously (Ekstrom et al. 2013). Briefly, liver tissue was collected from fetuses from legal abortions which were performed for socio-medical reasons at the Karolinska University Hospital between year 2000 and 2003. The studies were approved by the regional Ethics Committee in Stockholm and by the National Board of Health and Welfare. The fetal tissues were excised and immediately frozen in liquid nitrogen and stored at −70 within 2 h. The median gestation age of the fetuses was 10 weeks. The genders of the fetuses were unknown. The median maternal age was 29 years. None of the women reported any chronic or acute disease, regular drug use, or drug abuse.

Human adult liver specimens were collected from a human donor liver bank established at the Division of Clinical Pharmacology, Karolinska University Hospital, as described previously (von Bahr et al. 1980).

A pool of four human fetal liver cytosols and two adult pools from male liver tissue each including liver cytosols from three individuals were prepared and protein concentration was measured according to Lowry et al. (1951). Morphine (100 μmol/L) was incubated with the different cytosol pools (8 mg/mL protein equivalent) in Tris-HCl buffer (0.05 mol/L with 0.25 mmol/L $MgCl_2$) pH 7.4 and 0.05 mmol/L PAPS in a total volume of 125 μL. The reactions were incubated at 37°C for 25 min and were stopped by adding 125 μL ice-cold acetonitrile. After centrifugation at 4000*g* at 4°C for 15 min, the supernatant was removed and stored in −20°C prior to LC-MS/MS analysis.

Plasma sample preparation

Sample preparation was performed according to an earlier reported procedure (Andersson et al. 2012). In brief, 50 μL of plasma were precipitated with 100 μL acetonitrile containing the internal standards M3G-d_3, M6G-d_3, and M-d_3 (500 ng/mL). The supernatants were evaporated to dryness and reconstituted in 30 μL of 0.1% aqueous formic acid.

Urine sample preparation

Sample preparation was performed according to an earlier reported procedure (Andersson et al. 2013). In brief, 25 μL of urine were diluted fivefold with ultrapure water containing the internal standards (360 ng/mL).

Cytosol sample preparation

The cytosol supernatant was injected into the LC-MS/MS system. No internal standards were used.

Mass spectrometry (LC-MS/MS) method

The instrument used was an ACQUITY UPLC system connected to a XEVO TQ MS tandem mass spectrometer (Waters, Milford, MA). The analytical column used was an ACQUITY UPLC HSS T3, 2.1 × 100 mm, 1.8 μm particle size, kept at 60°C. A gradient elution was used using mobile phase A: 0.1% aqueous formic acid and mobile phase B: methanol. The flow rate for morphine and its metabolites was 0.2 mL/min. The total run time was 5.5 min and the injection volume was 3 μL.

The mass spectrometer was operated in the positive electrospray mode using selecting reaction monitoring (SRM) with MassLynx™/Target Lynx™ Software version 4.1 (Waters). The capillary voltage was 2 kV and the extractor voltage was set to 2 V. The source temperature was 120°C and the desolvation temperature was 350°C. The cone gas, N_2, flow was 50 L/h; desolvation gas, N_2, flow was 1000 L/h; and the collision gas, Ar, flow was 0.15 mL/min. Table 1 shows the individual settings for each analyte and the internal standards.

Table 1. The MS/MS acquisition parameters used in SRM mode for all analytes.

Analyte	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell time (sec)	Cone voltage (V)	Collision energy (eV)	Retention time (min)
M3S	366.16	286.19	0.077	42	24	1.80
M6S	366.16	286.19	0.077	42	24	2.46
M3G	462.23	286.17	0.025	46	30	1.51
M6G	462.23	286.17	0.025	46	30	2.05
M	286.22	152.86	0.025	48	40	2.10
M3G-d_3	465.36	289.10	0.03	44	32	1.50
M6G-d_3	465.36	289.10	0.03	44	32	2.04
M-d_3	289.34	201.07	0.025	44	25	2.11

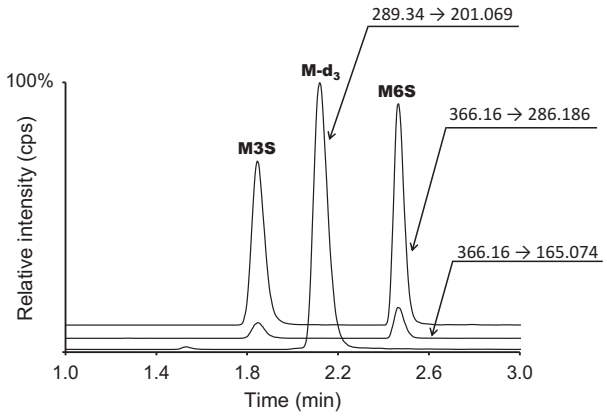

Figure 1. Calibration curves were generated between 14 and 14,000 nmol/L for the morphine sulfates. An LC-MS/MS chromatogram is displayed of a plasma calibrator spiked with 275 nmol/L of M3S and M6S, respectively.

Results

Validation of the method

A linear response was obtained between 14–14,000 nmol/L for M3S and M6S, 11–11,000 nmol/L for M3G and M6G, and 18–18,000 nmol/L in plasma with a correlation coefficient of >0.99 (n = 6) for all analytes. A chromatogram obtained from analysis of a 275 nmol/L plasma calibrator is shown in Figure 1. Lowest limit of quantification (LLOQ) were set to 11 nmol/L for M3G and M6G, 14 nmol/L for M3S and M6S, and 18 nmol/L for morphineM with a total CV of 7.3–19% (n = 25).

In urine, a linear response range was obtained between 100 and 20,000 nmol/L for all analytes. In an earlier validation made in urine, a linear calibration range was determined to 500–3,500,000 nmol/L for morphine, 300–900,000 nmol/L for M3G, and 300–130,000 nmol/L for M6G. LLOQ was set to 100 nmol/L with a total CV of 7.7–14.6% (n = 14–25). A summary of precision and accuracy data are shown in Tables 2 (plasma) and 3 (urine); calculated according to CLSI EP15-A2 (http://shopping.netsuite.com/s.nl/c.1253739/it.A/id.971/.f).

In human liver cytosol, a linear calibration response was obtained between 3 and 100 nmol/L for M3S and M6S.

Presence of M3S and M6S in vivo

M3S was present in plasma and urine from all studied patient groups (Table 4). Identification of M3S was based

Table 2. Accuracy, intra-assay, and total precision for all analytes at three concentrations in plasma..

Analyte	*n*	Conc. added (nmol/L)	Accuracy (%)	Intra assay CV (%)	Total precision CV (%)
M3G	25	30	110	3.6	5.0
	25	400	97	1.5	2.1
	25	6500	99	1.2	1.2
M6G	25	30	111	3.3	4.9
	25	400	98	1.6	1.6
	25	6500	96	1.6	1.6
Morphine	25	50	109	4.9	4.9
	25	500	100	2.7	2.7
	25	10,500	100	2.3	2.3
M3S	25	40	100	2.7	5.1
	25	500	84	1.3	6.8
	25	8000	99	2.0	3.6
M6S	25	40	104	2.3	3.6
	25	500	89	1.6	5.4
	25	8000	103	2.2	3.9

Calculations were made according to the CLSI EP15-A2.

Table 3. Accuracy, intra-assay, and total precision for all analytes at three concentrations in urine.

Analyte	*n*	Conc. Added (nmol/L)	Accuracy (%)	Intra assay CV (%)	Total precision CV (%)
M3G	25	300	97	5.0	5.0
	25	1100	99	2.4	3.2
	25	16,000	102	4.2	4.2
M6G	25	300	98	3.8	3.8
	25	1100	102	2.3	2.3
	25	16,000	102	4.0	4.0
Morphine	25	500	99	5.5	5.5
	25	1800	101	3.3	3.3
	25	26,000	101	4.4	4.4
M3S	25	400	94	3.3	4.9
	25	1400	92	3.4	3.4
	25	20,500	105	5.4	5.4
M6S	25	400	94	3.3	4.5
	25	1400	91	3.3	3.3
	25	20,500	104	4.1	4.1

Calculations were made according to the CLSI EP15-A2.

Table 4. A summary of the findings of morphine and its metabolites in patient samples

	M3S			M6S		M3G			M6G			mol/L		
Samples	*n*	nmol/L	Median	*n*	nmol/L	*n*	nmol/L	Median	*n*	nmol/L	Median	*n*	nmol/L	Median
Cancer patients plasma (n = 13)	6	14–150	22	0	–	13	30–20,000	1800	12	30–3000	260	11	40–5600	90
Cancer patients plasma M3S positive (n = 6)	6	14–150	22	0	–	6	4000–22,000	6000	6	250–3000	650	6	60–5600	500
Newborn patients plasma (n = 62)	17	14–60	25	1	20	36	13–650	180	32	10–200	60	35	20–500	120
Newborn patients plasma M3S positive (n = 17)	17	14–60	25	0	–	17	60–650	175	17	15–200	80	17	80–500	225
Cancer patients urine (n = 12)	9	145–8000	500	0	–	12	14,500–470,000	55,000	12	2400–250,000	16,000	11	350–200,000	5000
Cancer patients urine M3S positive (n = 9)	9	145–8000	500	0	–	9	23,000–470,000	144,000	9	3500–250,000	30,000	9	3000–200,000	6300
Drug testing urine (n = 196)	88	140–5000	275	1	160	161	140–380,000	70,000	155	140–200,000	15,000	172	200–27,000	7500
Drug testing urine M3S positive (n = 88)	88	140–5000	275	0	–	88	48,000–35,000	130,000	88	70,000–200,000	25,000	88	5000–270,000	25,000

on correct relative retention time (±0.5%) and ion ratio (±50%). Chromatograms for the plasma and urine samples are shown in Figure 2A and B. M3S was detected and quantified in about half of the plasma samples where M3G was present, that is, in 23 of 44 patients. In the cancer patient samples, the median value of M3G in M3S-positive samples was 6000 nmol/L (n = 6), while it was 650 nmol/L (n = 7) in M3S-negative samples. In samples from newborns, the median value of M3G in M3S-positive samples was 175 nmol/L (n = 17), while in M3S-negative samples it was 170 nmol/L (n = 44). The association of M3S being present with higher M3G plasma concentrations was only observed for the cancer patient group and not for newborns (Table 4).

In urine from cancer patient, M3S was present in 75% of the M3G-positive samples, while in samples collected for drug testing the corresponding value was 55% (Table 4).

M6S was observed only at a low concentration in one plasma sample from a newborn patient and in one sample in the urine drug testing group.

Calculations of the M3S/M3G ratio for all samples containing quantifiable concentrations of M3S are shown in

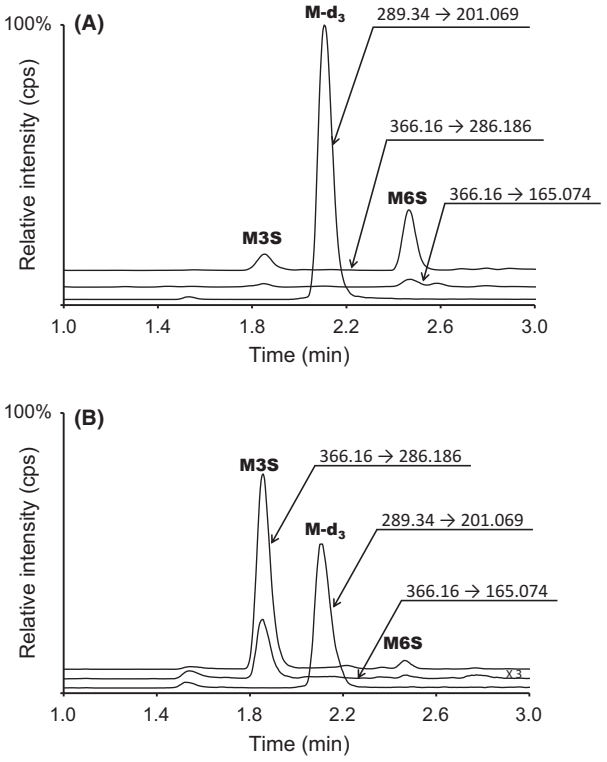

Figure 2. Analysis of M3S and M6S in authentic urine and plasma samples using LC-MS/MS. Chromatograms showed are (A) a plasma sample from the newborn group containing 20 nmol/L M6S and a detectable peak for M3S and (B) urine sample from the drug testing group containing 160 nmol/L M6S and 4000 nmol/L M3S.

Table 5. Calculations of the M3S/M3G ratio for all samples containing quantifiable concentrations of M3S.

	M3S/M3G %			
Study	*n*	%	Average	Median
Cancer patients plasma (*n* = 13)	6	0.29–0.54	0.41	0.41
Newborn patients plasma (*n* = 62)	17	2.66–24.1	12.8	12.7
Cancer patients urine (*n* = 12)	9	0.84–3.38	1.76	1.39
Drug testing urine (*n* = 196)	88	0.10–2.44	0.26	0.20

Table 5. In the newborn patient group a significantly higher mean value for the M3S/M3G ratio was observed as compared with the cancer patient group according to an unpaired *t*-test ($P < 0.05$). When plotting a second order polynomial graph comparing the gestational age to the M3S/M3G ratio for the newborn group a trend can be observed that preterm newborns have higher ratios than full-term newborns (Fig. 3).

Formation of M3S and M6S in vitro

The incubation of morphine with liver cytosol extracts resulted in the formation of both M3S and M6S. Representative chromatograms from an incubation of morphine with adult liver cytosol are shown in Figure 4. The formation rate of M3S was 0.17 nmol/mg protein per minute ($n = 8$) and for M6S the formation rate was 0.43 nmol/mg protein per minute ($n = 6$) in the adult liver cytosol using a substrate concentration of 100 μmol/L. These numbers corresponds to 0.0041% formation of M3S and 0.010% formation of M6S from morphine was observed

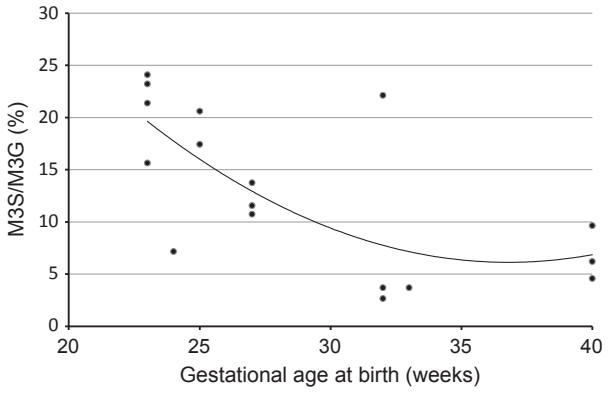

Figure 3. A second order polynomial graph displaying the M3S/M3G ratio for the newborn group compared to their gestational age at birth. A tendency can be observed that preterm newborns have higher ratios than full-term newborns.

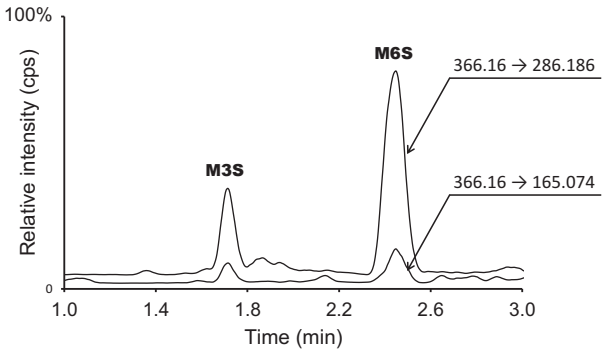

Figure 4. Formation of M3S and M6S in human liver cytosol was studied in vitro. An LC-MS/MS chromatogram is displayed of a morphine incubation with adult liver cytosol containing 20 nmol/L M6S and 8 nmol/L M3S.

in adult liver cytosol. In human fetal liver cytosol, the formation rate of M3S was 0.21 nmol/mg protein per minute ($n = 4$) and for the M6S formation the rate was 0.34 nmol/mg protein per minute ($n = 3$) using a substrate concentration of 100 μmol/L. The formation of M3S was substrate concentration dependent. Incubation using a low concentration of morphine (1 μmol/L) gave no detected product, while incubation at 1000 μmol/L gave 0.0035% formation of M3S using adult liver cytosol. Incubation using 100 μmol/L morphine for 15 or 35 min gave 1.7 times increase in M3S formation ($P < 0.05$). Data for M6S were not conclusive.

Discussion

The sulfation pathway of most drugs has not been thoroughly studied and for most drugs data of possible sulfate metabolites are lacking. An exception from this is acetaminophen (paracetamol) where it is known that approximately 25–35% of a given dose is metabolized by sulfation in adults and the rest by glucoronidation (McGill and Jaeschke 2013). Before this study was carried out it was not known how important the sulfation pathway was in morphine metabolism in humans.

Our main finding was that the human liver tissue can execute sulfation of morphine both at position 3 and 6, even in the newborn period. The in vitro studies revealed that M3S and M6S takes place in both in fetal and adult liver cytosol. The M3S metabolite was readily detected in samples from newborns, cancer patients, and heroin addicts. In addition, the present study demonstrates for the first time presence of morphine sulfated at position 6 (M6S) in blood and urine. However, in newborns M6S was found only in one of the 21 investigated newborns, and in heroin addicts it was measurable in one of 196 urine samples. No M6S was detected and in any sample from the 11 morphine-treated cancer patients.

Our results show that the metabolic conversion of morphine to M3S and M6S contributes only little to the overall conjugation of this drug. We estimated that less than 0.01% of a given morphine dose is converted into sulfate metabolites. An earlier attempt to detect M6S in human urine and plasma using an HPLC UV method was not successful (Choonara et al. 1990). The LC-MS/MS method used in the present study has approximately 25 times higher sensitivity enabling us to demonstrate formation of M6G, even though we found the metabolite in only two cases.

The clinical results were corroborated by our in vitro study of liver cytosol. Interestingly, the quantitative discrepancy between the two sulfate conjugates found in vivo was not observed in vitro. The rate of formation of M6S was comparable to that of M3S in the liver cytosols. There are manifold explanations for these, for example, differences in substrate concentrations at the enzyme level, cofactor availability, etc. In addition, other factors besides enzymology-related factors could explain the lack of detectable sulfate-metabolites in the circulation, such as the impact of active transport of metabolites out of hepatocytes in the in vivo situation.

The potential analgesic effect of morphine sulfates is of great interest. A number of derivatives have been prepared and studied as drug candidates (Houdi et al. 1996; Crooks et al. 2006). This was one reason for this detailed study of M3S and M6S. Most focus as candidate for pharmacological activity has been put on M6S. However, despite the fact of higher potency of M6S compared to morphine, the very low concentrations of M6S makes it unlikely that M6S contributes substantially to the analgesic effect of the parent drug. This is in agreement with the conclusion made before (Choonara et al. 1990).

As expected, the concentration levels of M3S were much lower than for M3G. The M3S/M3G ratio gives an estimate of the relative importance of these metabolic pathways. This ratio was higher in newborns than in cancer patients indicating that the relative importance of sulfation decreases during ontogeny whether this be caused by a decrease or an increase in the respective pathways. This is expected since sulfation is a more important pathway in fetal life and early infancy (Besunder et al. 1988). The ontogenic metabolic pattern of acetaminophen (paracetamol) provides a similar and conspicuous example of such a developmental switch from sulfation to glucuronidation (McGill and Jaeschke 2013). We found no preference for the sulfation pathway in the terminal cancer patients.

This study had several strengths. First, due to the sensitivity of our new method we could detect M6S for the first time. Second, we were able to study the development of morphine sulfation in humans at the extremes of age, namely in human fetuses and newborns, and in adults in terminal care. Finally, with the sensitive LC-MS/MS method conclusive identifications could be made due to the available reference material of M3S and M6S.

One limitation of this study is the rather small number of newborns and cancer patients included. Larger patient materials are needed to characterize the true metabolic pattern and its ontogeny.

Since only one of the newborns and one of the heroin addicts had detectable M6S, it could be speculated that the formation of this metabolite might be more pronounced in some individuals. Whether this may be ascribed to genetic polymorphisms in any of the involved SULT remain to be studied. Genetic variations in SULT1A2 and 2A1 have been associated with alteration in drug metabolism of tamoxifen, salbutamol, as well as androgenic steroids (Boulton and Fawcett 2001; Gjerde et al. 2008; Schulze et al. 2013).

In conclusion, sulfation of morphine is present in human liver but this metabolic pathway seems to be of minor importance in vivo and probably lacks clinical significance. The M6S metabolite is formed in a very low amount, making it undetectable in most individuals.

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Disclosures

None declared.

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