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Postmortem blood and tissue concentrations of R- and S-enantiomers of methadone and its metabolite EDDP

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Abstract

Aim: Methadone (MTD) is frequently used for treatment of opiate addiction. S-MTD shows analgesic effects only in large doses, whereas R-MTD is mainly responsible for pharmacological effects. MTD clearance is primarily attributed to CYP3A4 and CYP2B6, and metabolism of the racemic compound is highly stereoselective. Aim of the present study was to investigate the enantiomeric ratios of MTD and 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in postmortem body fluids and tissues after administration of both racemic and enantiomerically pure R-MTD. It had to be established if R-MTD accumulates in body fluids and tissues after repeated dosing. Furthermore, mean femoral blood concentrations and R/S-ratios of MTD and EDDP of cases participating in methadone maintenance therapy (MMT) and non-MMT cases have been compared.

Methods: R- and S-MTD as well as R- and S-EDDP concentrations were determined by chiral LC-MS/MS following liquid-liquid extractions of body fluids and tissue homogenates of 16 MTD-related fatalities.

Results and discussion: R/S ratios of MTD and EDDP in femoral blood ranged from 0.97 to 3.88 for MTD and from 0.68 to 1.35 for EDDP, respectively. R-MTD appeared to accumulate in all media after repeated dosing. Mean femoral blood concentrations of MTD and EDDP and mean femoral blood R/S ratio of MTD and in MMT cases were significantly higher than in non-MMT cases, whereas no difference of the mean femoral blood R/S ratios of EDDP could be observed in both groups.

Conclusion: Accumulation of R-MTD might be due to repeated dosing and its longer mean half life compared to S-MTD. The enantiomeric ratios of MTD and EDDP are useful for the interpretation of postmortem concentrations and to differentiate between the consumption of racemic or enantiomerically pure R-MTD.

1. Introduction

Methadone (RS-dimethylamino-4,4-diphenylheptan-3-on, MTD), a synthetic diphenyl-propylamine, is frequently used for treatment of opiate dependent persons by qualified physicians. In Germany, leakage of MTD onto the black market occurs e.g. from take-home prescription [1]. Therefore, intoxication may also occur in an individual who does not participate in a methadone maintenance therapy (MMT).

Although MTD's structure differs from that of morphine, it has clinically comparable actions and analgesic effects. Its metabolism preferably occurs in the liver and is catalyzed by cytochrome-P450 enzymes such as CYP2B6 [2]. In addition, CYP2C19 has also been reported to metabolize MTD. The primary metabolic route is CYP2B6-catalyzed *N*-demethylation to the pharmacologically inactive 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) [3]. According to literature, metabolism of MTD shows individual stereoselective variability [4]. CYP2B6, which reportedly plays a dominant role in MTD metabolism, *N*-dealkylates S-MTD

at higher rates than R-MTD. In contrast, CYP2C19 shows opposite stereoselectivity. Also, CYP3A4 activity has a modest effect on MTD-*N*-demethylation which is not stereoselective [2,3,5].

During MMT, the drug is usually administered once daily with respect to its long half-life via the oral route in maintenance doses of 80-100 mg starting with an initial dose of 20-40 mg. Target plasma concentrations are recommended to be 400 ng/mL for racemic MTD or 250 ng/mL for R-MTD [6]. Especially in the initial phase of MMT there is an increased risk of side effects such as respiratory depression or cardiac rhythm disorders. Poor health as well as the use of other drugs such as heroin and benzodiazepines are likely to increase the risk of adverse and toxic effects [6].

S-MTD shows analgesic effects only in large doses, whereas pharmacological activity is due almost entirely to R-MTD [7]. For R-MTD has a longer elimination half-life (mean: 38 h) compared to S-MTD (mean: 29 h) [6], stereoselective metabolism may influence its blood level resulting in an increased or decreased duration of action. Especially in fatalities in maintenance patients (MMT cases), stereoselective metabolism may contribute to death by accumulation of the active R-enantiomer. Therefore, aim of the present study was to investigate the enantiomeric ratios of MTD and EDDP in postmortem body fluids and tissues after administration of both racemic MTD and enantiomerically pure R-MTD.

2. Material and Methods

2.1. Experimental Design

Depending on availability, samples of blood from the femoral vein and the heart, gastric contents, bile, urine, cerebrospinal fluid (CSF) as well as specimens taken from the liver, lungs, kidneys, muscle and brain were provided from all MTD-related deaths not involving further opioids during 2011 and 2012. A number of 16 cases could be investigated, including eleven males and five females with a mean age of 31 years (23-42 years). Fifteen of them had a known history of drug misuse or drug dependence. Prior to death, eight of the deceased had participated in a MMT.

After routine toxicological investigations were completed, MTD and EDDP were extracted by a standard liquid/liquid extraction method: borate buffer pH 8.5, deuterated internal standards and ethyl acetate were added to100 µL of body fluids or 100 mg of tissues as well as the corresponding calibration standards. Samples were vigorously shaken and centrifuged. The organic layer was evaporated to dryness. The residues were reconstituted in 100 µL mobile phase.

Enantiomeric separation of MTD and EDDP was performed on an LC-MS/MS consisting of an Agilent Series HPLC apparatus (Agilent, Waldbronn, Germany) and an API 4000 tandem mass spectrometer (AB Sciex, Darmstadt, Germany) with TurboIon^{TM} ionization source operating in the positive ionization mode. A Chiral AGP column (ChromTech, Apple Valley, USA) was used as stationary phase. The mobile phase consisted of 5 mM ammonium acetate buffer pH 4.1 and acetonitrile in a ratio of 98:2 by vol. A detailed description of materials and method is given in reference [8].

2.2. Evaluation of the Analytical Method

The method was validated according to the current guidelines of the GTFCh (Gesellschaft für Toxikologische und Forensische Chemie) [9], which are in line with international guidelines. Validation data are given in reference [8].

2.3. Statistical Analysis

A t-test was performed to test whether MTD and EDDP concentrations as well as MTD- and EDDP-R/S-ratios in femoral and heart blood were significantly different or not (Microsoft Excel). A p-value below 0.05 was considered significant.

3. Results and Discussion

R-MTD and R-EDDP could be detected in all body fluids and tissues under investigation. The respective S-enantiomers were present in 13 cases, whereas in cases #2, #3 and #15, R-MTD and R-EDDP could exclusively be detected in all specimens.

Total MTD concentrations ranged from 225-3,271 ng/mL in femoral and heart blood, whereas total EDDP concentrations were from 21-2,481 ng/mL. Tab. 1 summarizes the enantiomeric and total femoral blood concentrations of MTD and EDDP in all cases under investigation. For details in tissue distribution of the enantiomers of MTD and EDDP, see Tab. 2 and 3.

Tab. 1. R-, S- and total MTD as well as R-, S- and total EDDP femoral blood concentrations of all cases under investigation. nd: not detected; na: not available

Case #	R-MTD (ng/mL)	S-MTD (ng/mL)	total MTD (ng/mL)	R-EDDP (ng/mL)	S-EDDP (ng/mL)	total EDDP (ng/mL)
1	1,076	277	1,353	1,220	1,261	2,481
2	256	nd	256	23	nd	23
3	567	nd	567	64	nd	64
4	316	219	535	25	37	62
5	na	na	na	na	na	na
6	142	113	255	22	24	46
7	188	193	381	17	15	32
8	345	163	508	42	31	73
9	1,401	801	2,202	45	50	95
10	389	218	607	24	28	52
11	147	127	274	16	16	32
12	269	191	460	23	27	50
13	744	540	1,284	24	28	52
14	2,034	1,237	3,271	59	48	107
15	268	nd	268	21	nd	21
16	182	178	360	17	16	33

In cases where both enantiomers were present, the mean R/S ratio ranged from 0.98-3.96 for MTD and from 0.61-1.18 for EDDP. The intra-subject variability was from 5.1-18.8 % for MTD and from 8.8-23.2 % for EDDP, respectively. For details, see Fig. 1.

In femoral blood, the mean MTD R/S-ratio of MMT cases (n=6) was 1.69 (range 1.41-2.12). In contrast, the mean MTD R/S-ratio of cases without treatment monitoring (n=6) was 1.16 (range 0.97-1.38). For EDDP, mean R/S-ratios were 0.98 (range 0.68-1.35) for MMT-cases and 0.99 (range 0.86-1.13) for non-MMT cases, respectively. Case #1 being an outlier has been excluded from data analysis.

Tab. 2. Ranges and medians of R-MTD, S-MTD and total MTD concentrations in body fluids (ng/mL) and tissues (ng/g); CSF: cerebrospinal fluid, * total concentration (µg), n: number of samples with results ≥ LLOQ.

Analyte		R-MTD			S-MTD			total MTD	
Material	**n**	**range**	**median**	**n**	**range**	**median**	**n**	**range**	**median**
Femoral blood	15	142-2,034	316	12	113-1,237	206	15	255-3,271	508
Heart blood	16	116-2,171	616	13	109-1,045	604	16	225-2,775	1152
Brain	15	157-1,577	412	12	158-1,075	312	15	219-2,652	677
Liver	15	201-4,134	625	12	184-1,658	472	15	276-5,127	1199
Lungs	15	508-23,250	3365	12	525-10,500	2934	15	1033-29,005	6582
Kidneys	15	195-2,058	470	12	147-1,424	394	15	338-3,482	884
Muscle	15	94-785	276	12	86-483	195	15	152-1,268	432
Fat	6	61-1,308	268	6	57-910	227	6	118-2,218	494
Urine	13	390-52,878	2503	10	275-39,670	3196	13	665-92,548	3824
Bile	15	699-7,165	1961	12	825-3,700	1340	15	699-10,865	2975
Gastric contents*	14	16-1,033	136	11	13-1,530	107	14	29-2,410	286
CSF	8	81-426	183	5	98-437	174	8	81-863	229

Tab. 3. Ranges and medians of R-EDDP, S-EDDP and total EDDP concentrations in body fluids (ng/mL) and tissues (ng/g) under investigation. CSF: cerebrospinal fluid, * total concentration (ng), n: number of samples with results ≥ LLOQ.

Analyte		R-EDDP			S-EDDP			total EDDP	
Material	**n**	**range**	**median**	**n**	**range**	**median**	**n**	**range**	**median**
Femoral blood	15	16-1,220	24	12	15-1,261	28	15	21-2,481	52
Heart blood	16	16-1,117	27	13	16-1,208	35	16	21-2,325	58
Brain	15	3-76	10	12	11-82	12	15	3-158	22
Liver	15	12-2,564	29	12	15-2,453	36	15	15-5,017	57
Lungs	15	13-3,211	19	12	15-3,610	23	15	13-6,821	40
Kidneys	15	16-4,431	52	12	18-4,610	56	15	21-9,041	117
Muscle	15	10-1,757	15	12	13-1,683	17	15	12-3,440	33
Fat	6	10-266	21	6	12-207	21	6	22-473	41
Urine	13	145-7,440	318	10	120-2,817	403	13	280-7,440	690
Bile	15	480-58,470	1605	12	580-50,270	1695	15	845-108,740	3715
Gastric contents*	12	375-379,000	4200	10	365-351,000	2750	12	740-730,000	7750
CSF	7	4-24	5	5	4-5	5	8	5-24	10

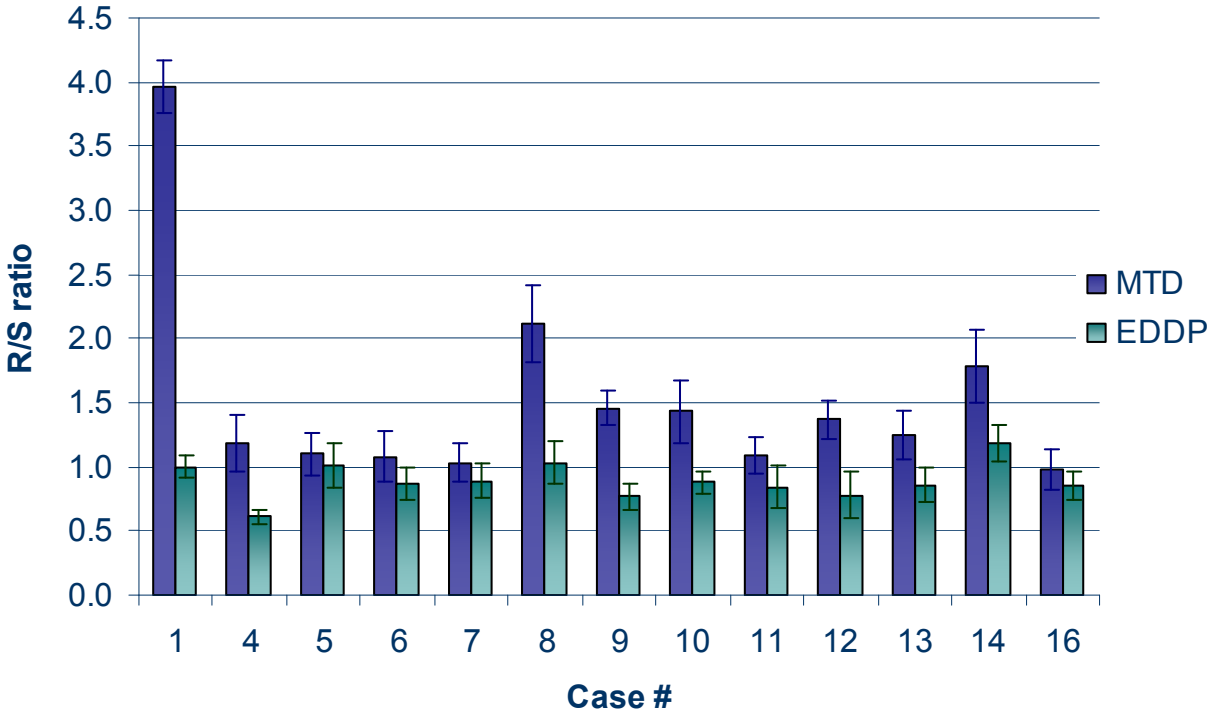

Fig. 1. Mean R/S enantiomeric ratios and standard deviations of MTD and EDDP in body fluids and tissues of MTD-related postmortem cases.

Showing a span of femoral blood concentrations of 255-3,271 ng/mL, the total MTD concentration determined in all 16 cases always exceeded 200 ng/mL. These results are in line with literature data of MTD concentrations in fatalities [10-16]. The R-MTD concentrations determined from cases #2 and #15 and the total MTD concentrations of all cases except cases #1, #9, #13 and #14 were well within a concentration range considered therapeutic in living patients [7]. Foster et al. [17] found plasma R-MTD and S-MTD peak concentrations ranging from 120-362 ng/mL and 116-447 ng/mL in 18 MTD patients. De Vos et al. [18] observed far higher maximum plasma concentrations of 124-1,255 ng/mL in opiate addicts participating in a MMT. A reference range of R-MTD during MTD therapy from 40-300 ng/mL has been given; nevertheless, a concentration of 200 ng/mL has been attributed to fatal overdosage as well [11].

Both R- and S-MTD as well as R- and S-EDDP could be detected in 13 out of the 16 cases with MTD R/S-ratios of 0.97-3.88 (mean 1.65, median 1.43) being considerably higher than R/S-ratios of EDDP of 0.67-1.35 (mean 0.98, median 0.95) determined from femoral blood. As in the present study, R/S-ratios of MTD in femoral blood greater than 1.0 have also been observed in 10 fatalities by Johansen and Linnet [19] ranging from 1.00-2.62. In the same study, R/S EDDP ratios were estimated to range from 0.60-1.50. Holm and Linnet [20] determined MTD and EDDP R/S ratios of 1.12-2.38 and 0.59-1.10 in femoral blood samples from six postmortem cases, respectively.

The present results are in agreement with the findings of Drasch et al. [21], who reported on R/S-ratios of MTD in 106 postmortem blood samples ranging from 0.32-3.48. Their mean R/S ratio being 1.19 and therefore lower than in the present study is presumably due to the higher number of samples.

From cases #2, #3 and #15, where only R-EDDP and R-MTD could be detected in all specimens, it can be assumed that MTD is not prone to *in vivo* racemization as it has been described for drugs such as ibuprofen and thalidomide [22]. R/S-ratios of MTD and EDDP in

case #1 significantly differ from each other. With respect to a survival time of about 24 h following last drug administration by the deceased himself similar R/S ratios of both compounds should have been expected. A plausible explanation might be administration of R-MTD in the hospital close to the time of death to avoid withdrawal symptoms. MTD *in vivo* racemization as an explanation can be precluded due to findings from cases #2, #3 and #15.

Statistical analysis revealed significant differences ($p < 0.05$) between the mean MTD R/S-ratios of MMT cases (n=6) and those without therapy (n=6) in femoral blood. In addition, the mean MTD R/S-ratio of 1.69 from femoral blood of MTD patients exceeds that from living patients (n=7) receiving daily doses of 5-100 mg MTD, where a mean MTD R/S-ratio of 1.39 has been observed [23].

Overall, mean MTD R/S-ratios in body fluids and tissues appear to be consistent within but different between respective groups. For example, the mean femoral blood concentration of the MMT-group of 1,264 ng/mL was considerably higher than that of 511 ng/mL of the non-MMT group. These results are in line with the findings of Gagajewski and Apple [24], who observed a mean MTD concentration of 1,140 ng/mL in blood samples from the vena cava inferior of MMT cases compared with a mean concentration of 820 ng/mL in illicit users following accidental overdose. With respect to MTD R/S-ratios in the MMT-group, accumulation of R-MTD due to regular daily dosage may be a likely explanation for the higher MTD concentrations in these individuals and may be attributed to the different elimination half-lives of the enantiomers of 37.5 h for R-MTD and of 28.6 h for S-MTD [25]. Co-consumption of illicit MTD during MMT might favor accumulation of the R enantiomer; according to Waldvogel et al. [26], injection of illicit MTD during MMT is not uncommon. Also, as reported by Judson et al. [27], 18.5 % of MMT patients had co-administered illicit MTD by the intravenous route. It may be speculated whether the non-MMT group had limited and infrequent access to MTD; thus, accumulation of R-MTD is suggested to be less likely.

In contrast to MTD, mean EDDP R/S-ratios did not differ between groups. In MMT subjects, the mean EDDP R/S-ratio in femoral blood was 0.98 (range 0.68-1.35), whereas in the non-MMT group, the mean ratio was 0.99 (range 0.86-1.13). EDDP R/S-ratios of both groups did not differ significantly for both femoral and heart blood.

4. Conclusions

Enantioselective analysis of MTD is considered to be a useful tool for the interpretation whether a postmortem concentration is actually in a therapeutic or potentially toxic range because the concentration of the pharmacologically active R-MTD provides a more specific measure of impairment. Obviously, *in vivo* racemization does not occur. Therefore, the enantiomeric ratio of EDDP might be useful to evaluate if there was a recent consumption of either R-MTD or racemic MTD, especially if R/S-ratios of EDDP and MTD significantly differ. Also, S-MTD or S-EDDP findings are indicative of administration of racemic MTD. Enantiomeric ratios of MTD differed between cases with a history of MMT and those not participating in MMT; accumulation of R-MTD due to regular dosing may be a likely explanation.

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