Journal of Chromatography A, 1218 (2011) 4988–4994

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Distribution of metoprolol, tramadol, and midazolam in human autopsy material

R. Oertel[a,*], J. Pietsch[b], N. Arenz[a], S.G. Zeitz[b], L. Goltz[a], W. Kirch[a]

[a] Institute of Clinical Pharmacology, Medical Faculty Carl Gustav Carus, Technical University Dresden, Fiedlerstr. 27, 01307 Dresden, Germany
[b] Institute of Legal Medicine, Medical Faculty Carl Gustav Carus, Technical University, Dresden, Germany

ARTICLE INFO

Article history:
Available online 5 January 2011

Keywords:
Drug
Distribution
Compartment
Autopsy
Human
LC/MS/MS

ABSTRACT

In this study it was possible to measure the distribution of metoprolol, tramadol, and midazolam in human directly in several compartments. In the legal medicine autopsy material is normally investigated to find out the cause of death. But the results of corresponding toxicology measurements often involve more information. With screening methods drugs were detected without connection to the cause of death. The deceased had either a continual therapeutic treatment, a treatment during an operation, or an unsuccessful urgent therapy. A liquid/liquid extraction and a LC/MS/MS method were developed for the determination of the drug concentrations. Different autopsy materials of about 120 cases were investigated. Most frequently the drugs metoprolol, tramadol, and midazolam could be proved and determined simultaneously. Metoprolol was found in seven cases, tramadol in seven cases and midazolam in thirteen cases. The dosage of the drugs was unknown. Therefore and because of the low number of cases statistic calculations were not meaningful and an individual case study was necessary. In all cases with oral metoprolol application the patients probably took a normal customary continuous dosage. The concentrations of tramadol in blood were in the toxic range in three cases. The distribution of tramadol in the compartments was independent of the dosage. The time between oral intake of metoprolol or tramadol and death was unknown. With the distribution pattern of metoprolol in the compartments it was possible to estimate the duration between drug intake and death. In most cases midazolam was given intravenously during an operation or an unsuccessful urgent therapy. Sometimes the time between dosage and death was documented. The duration between application of the drug and death played the crucial role for the distribution of midazolam in the compartments. Measurements of drug concentrations in human autopsy material deepen the knowledge of the respective drugs' pharmacokinetics.

1. Introduction

In the legal medicine autopsy material is normally investigated to find out the cause of death. But the results of corresponding toxicology measurements often involve more information. With screening methods in many cases drugs were detected without connection to the cause of death. The deceased had either a continual therapeutic treatment, an administration in the course of an operation, or an unsuccessful urgent therapy. If a drug was identified in autopsy material, the sample was analyzed again after standard addition in this study.

Most frequently the drugs metoprolol, tramadol and midazolam could be proved and determined in different autopsy materials. Drug concentrations in autopsy material after therapeutic dosage were reported for the first time.

Metoprolol, a β1-selective adrenoceptor antagonist, is widely used in the treatment of mild to moderate hypertension and angina pectoris. Blockade of the β1 receptor reduces heart rate, myocardial contractility and cardiac output. Metoprolol reduces plasma renin activity [1,2]. Numerous causes with metoprolol intoxication have been published. Distribution of metoprolol and its post-mortem redistribution in rabbits are described. But animal experiments have a limited meaningfulness [3–7].

Tramadol is a synthetic, centrally acting analgesic agent with two distinct, synergistic mechanisms of action, acting as both a weak opioid agonist and an inhibitor of monoamine neurotransmitter reuptake. Tramadol is available as drops, capsules and sustained-release formulations for oral use, suppositories for rectal use and as solution for intramuscular, intravenous and subcutaneous injection [8,9]. There are several published cases of tramadol's involvement in drug-related deaths and impairment. Reports of deaths involving tramadol associated with tissue concentrations are rare [10–15].

Midazolam is a short-acting benzodiazepine with anxiolytic, sedative-hypnotic and marked amnestic properties. Due to an excellent local tolerability and its slight reduction in blood pressure and minor dose-related respiratory depression, midazolam is useful for anaesthetic induction and postoperative (long-term)

* Corresponding author. Tel.: +49 3514584534; fax: +49 3514584341.
E-mail address: reinhard.oertel@tu-dresden.de (R. Oertel).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.12.113

Table 1
Mass spectrometer conditions.

Substance	Molecular weight	Precursor ion	Product ion	Cone voltage [V]	Collision energy [eV]	Dwell time (s)
Metoprolol	267.36	268.0	116.0	24	18	0.15
Tramadol	263.38	264.4	57.8	22	15	0.15
Midazolam	325.77	326.0	291.3	34	14	0.15

sedation especially for intensive care patients [16–19]. Reports of midazolam concentrations in tissues are rare [20,21].

In this study it was possible for the first time to measure the distribution of metoprolol, tramadol or midazolam in human simultaneously and directly in several compartments using a newly developed and validated LC/MS/MS screening method. Similar LC/MS/MS methods for the individual drugs have been reported [22–26].

2. Experimental

2.1. Chemicals

Metoprolol, tramadol, and midazolam were purchased from Sigma (St. Louis, MO, USA). In addition dichloromethane LiChrosolv (for chromatography), acetonitrile LiChrosolv (for chromatography), methanol LiChrosolv (for chromatography), formic acid (p.a.) and ammonium acetate (p.a.) were purchased from MERCK (Darmstadt, Germany). Finally pure water (18 MΩ) was obtained using an ion exchange system RS 40 E, SG Ionenaustauscher (Barsbüttel, Germany).

2.2. LC–MS–MS analysis

The LC/MS/MS system used was a Quattro Micro (Micromass, Manchester, UK) equipped with an electrospray interface. Full scan mass spectra were acquired by continual infusion of standard solution (concentration 100 ng/ml at 10 μl/min) and by scanning MS1 from *m/z* 150 to *m/z* 350. The product ion mass spectra were obtained by choosing the protonated molecule $[M+H]^+$ as the precursor ion and scanning MS2 from *m/z* 80 to *m/z* 350 with argon as collision gas (pressure 3.5×10^{-3} mbar). The capillary voltage was 3.500 V (positive ion mode) and an ion source temperature of 100 °C was applied.

The drugs were measured using the multiple reaction monitoring mode (MRM) with the specific transitions between *m/z* 268.0 (precursor ion) and *m/z* 116.0 for metoprolol, between *m/z* 264.4 (precursor ion) and *m/z* 57.8 for tramadol, and between *m/z* 326.0 (precursor ion) and *m/z* 291.3 for midazolam. The cone voltage, collision energy and dwell time are shown in Table 1. The desolvation gas flow (nitrogen) was 600 l/h at 300 °C.

The HPLC equipment consisted of a Dionex P680 HP-gradient pump and an autosampler Dionex UltiMate 3000 ACC (Idstein, Germany) with a Chromeleon Chromatography Data System (Dionex Softron, Idstein, Germany). The chromatographic separation was performed on a Synergy 4μ Polar-RP 80A, 150 mm × 2 mm (Phenomenex, Aschaffenburg, Germany) column with a Security Guard C18, 4 mm × 2 mm I.D. (Phenomenex, Aschaffenburg, Germany).

The following mobile phase gradient was applied with solvent A (5/95/0.2, v/v/v) and solvent B (95/5/0.2, v/v/v) of a mixture of acetonitrile, 2 mM ammonium acetate and formic acid: 0–0.6 min 100% (v/v) solvent A; 0.6–2.0 min a steep linear gradient was programmed to 0% (v/v) solvent A; 1.8 min 0% (v/v) solvent A; 3.8–4.0 min a steep linear gradient to 100% (v/v) solvent A; 4.0–7.3 min 100% (v/v) solvent A.

The column temperature and flow rate were 35 °C and 0.4 ml/min, respectively. The retention times of metoprolol, tramadol, and midazolam were 3.6 min, 3.7 min, and 3.9 min, respectively.

The MassLynx 4.0 data system (Waters) was applied for MS control and QuanLynx 4.0 (Waters) for the peak area evaluation, regression analysis of standard curves and calculation of concentrations.

2.3. Sample preparation and standard addition

A liquid–liquid extraction procedure was used for sample preparation. The first step was to screen a blood or urine sample from each autopsy with a HPLC-UV method for drugs (not shown). If a drug was found its concentration was estimated. Then the autopsy samples were prepared twice: pure and spiked with the proved drug for quantification by standard addition procedure. The amount of the added standard corresponded to the estimated drug concentration.

0.5 ml of heart blood, venous blood, and urine as well as about 0.5 g of intestines (brain, liver, and kidney) were alkalised with 100 μl of ammonia buffer (pH 9). Samples were extracted with 1.0 ml of dichloromethane. After vortexing and centrifugation the eluates were evaporated to dryness in a stream of nitrogen at 50 °C, redissolved in 500 μl of mobile phase mixture, and 20 μl were injected for LC–MS–MS.

2.4. Calculation

Standard addition procedure was used instead of an external calibration curve to solve the recovery and the matrix effect problems. A solution of known concentration of metoprolol, tramadol or midazolam (for example 0.50 μg/ml) was added to the unknown autopsy samples. Samples were extracted and analyzed with and without standard addition. The two readings – before and after adding the standard – were used to extrapolate and determine the concentration initially in the unknown sample. Additional standard curves containing metoprolol, tramadol and midazolam in mobile phase mixture in the range from 7.8 to 1000 ng/ml were measured directly without sample preparation. These standard curves were used for quality control and for estimation of the recovery of the drugs.

2.5. Case reports

Seven cases (Me1–Me7) with metoprolol and seven cases with tramadol (T1–T7) in autopsy material were discovered. These and the thirteen case reports with determined midazolam (Mi1–Mi13) in the autopsy material are shown in Table 2. In all cases the dosage of the drugs was not given in the documents.

3. Results and discussion

3.1. Quantification and recovery

The calibration graphs were generated from MRM of increasing amounts of standards in mobile phase mixture. A quadratic calibration graph was constructed using least-squares regression analysis of quantities versus peak area. A good response over the range of 7.8–1000 ng/ml was demonstrated. The correlation coef-

Table 2
Case reports with metoprolol (Me), tramadol (T) or midazolam (Mi) in autopsy material.

No.	Age [years]	Weight [kg]	Sex	Cause of death	Medication	Application	Time[a] [min]
Me-1	70	96	Female	Craniocerebral trauma (accident)	Continuous	Oral	w.g.
Me-2	57	76	Female	Intoxication with insuline and heart failure	Continuous	Oral	w.g.
Me-3	58	w.g.	Female	Intoxication with ethanol, doxylamine and temazepam	Continuous	Oral	w.g.
Me-4	81	70	Female	Embolism	Continuous	Oral	w.g.
Me-5	75	81	Male	Intestinal bleeding to death	Continuous	Oral	w.g.
Me-6	57	w.g.	Male	Myocardial infarct	Continuous	Oral	w.g.
Me-7	48	96	Male	Heart failure	Emergency	Intravenous	30
T-1	43	87	Female	Heart failure left	Continuous	Oral	w.g.
T-2	60	75	Male	Intoxication with tramadol and carbamazepine	Continuous	Oral	w.g.
T-3	37	74	Male	Intoxication with tramadol	Continuous	Oral	w.g.
T-4	52	108	Female	Heart failure right	Continuous	Oral	w.g.
T-5	73	74	Male	Organ failure	Continuous	Oral	210
T-6	25	45	Female	Intoxication with tramadol and doxylamine	w.g.	Oral	60
T-7	64	75	Male	Organ failure	Continuous	Intravenous	w.g.
Mi-1	42	90	Male	Polytrauma (accident)	Emergency	Intravenous	30
Mi-2	43	94	Male	Heart failure left	Emergency	Intravenous	w.g.
Mi-3	83	103	Female	Cardiac insufficiency	Emergency	Intravenous	w.g.
Mi-4	81	87	Male	Aneurysm of the aorta	Emergency	Intravenous	30
Mi-5	67	59	Female	Polytrauma (accident)	Emergency	Intravenous	w.g.
Mi-6	86	86	Male	Polytrauma (accident)	Emergency	Intravenous	w.g.
Mi-7	76	84	Male	Fat embolism	Emergency	Intravenous	w.g.
Mi-8	18	78	Male	Virus myocarditis	Emergency	Intravenous	45
Mi-9	65	115	Male	Organ failure	Emergency	Intravenous	50
Mi-10	71	87	Female	Hypoxia	Emergency	Intravenous	w.g.
Mi-11	29	69	Male	Cardiac infarction	Emergency	Intravenous	w.g.
Mi-12	73	74	Female	Organ failure	Emergency	Intravenous	w.g.
Mi-13	35	72	Male	Death by hanging	Continuous	Oral	<180

w.g., without giving.
[a] Time—time between application and death.

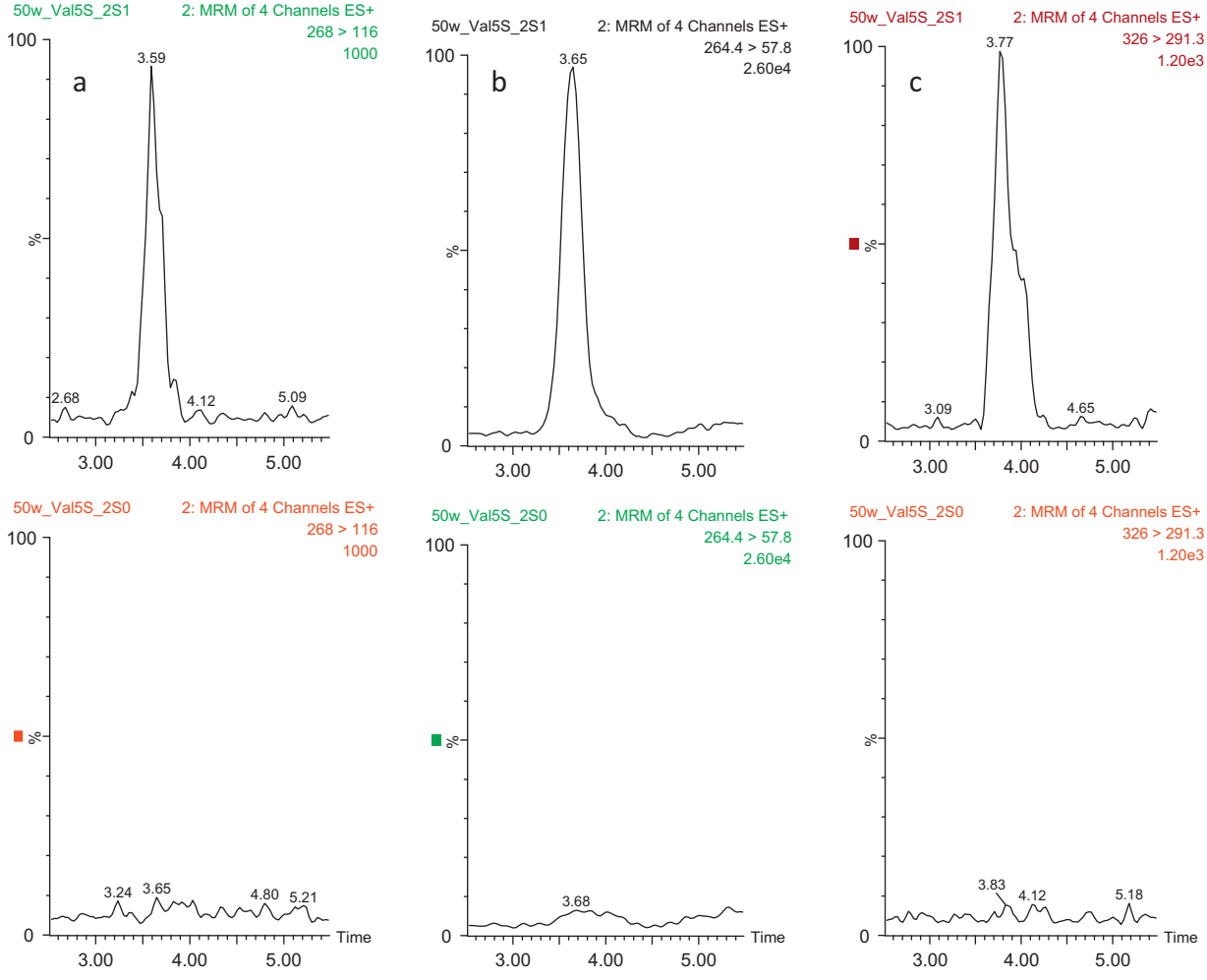

Fig. 1. MRM chromatograms for (a) metoprolol, (b) tramadol, and (c) midazolam—below blank sample and above standard with 7.8 ng/ml (LLOQ).

ficient of the regression lines was 0.9997 or higher. Precision and accuracy of the method were assessed by the determination of six concentrations in six independent series of spiked samples. For the analytes the precision was better than 5.0% (with the exception on the limit of quantification 8.6%) and the accuracy ranged from 97.0% to 106.4% of the respective nominal values. The lower limit of quantification, i.e. the lowest point of the standard curve with a coefficient of variation less than 10.0% for 6 repeated measurements, was 7.8 ng/ml (Fig. 1a–c). Samples with drug concentrations >1000 ng/ml were diluted.

The recoveries of the drugs after liquid–liquid extraction were calculated with the ratio of measured standard concentration and added standard concentration. The mean recoveries were 65% for metoprolol, 64% for tramadol, and 51% for midazolam.

3.2. Metoprolol concentrations

The concentrations of metoprolol in different body liquids and tissues are given in Table 3. Physico-chemical properties of a drug are important for its distribution in the body. Metoprolol has a partition coefficient KP = 0.16 and a dissociation constant pK_a = 9.68. Orally administered metoprolol is almost completely absorbed, although first-pass metabolism reduces its systemic availability by about 50%. Lipophilicity and a low degree of binding to plasma proteins facilitate extensive distribution (volume of distribution V_d = 4 L/kg), and penetration into the central nervous system. Therapeutic plasma levels of metoprolol are 0.02–0.34 μg/ml. After extensive hepatic metabolism via cytochrome P450 (CYP2D6) metoprolol is excreted primarily as inactive metabolites, about 95% of a dose is recovered in the urine within 72 h (approximately 3% as unchanged drug). Total body clearance ranges between 43.2 and 92.4 L/h and the elimination half-life is usually 3–4 h [1,2,27–29]. In the seven discovered cases intoxication with metoprolol could be excluded.

3.2.1. Metoprolol concentrations after oral therapeutic dosage

The metoprolol concentrations in blood were below 250 ng/ml. One can assume that the metoprolol tablets were taken regularly in customary dosage. The time between oral intake and death and the kind of tablets (conventional or slow release formulations) are unknown. Therefore and because of the low number of cases statistic calculations were not meaningful and an individual case study was necessary.

After metoprolol is swallowed, it is absorbed by the digestive system and enters the hepatic portal system. It is carried through the portal vein into the liver before it reaches the rest of the body. The liver metabolizes metoprolol, only a part of the active drug emerges from the liver into the rest of the circulatory system. The drug concentration in the liver is high a short time after oral intake. Afterwards a steady state of absorption, distribution, and elimination of the drug appears. The absorption and distribution of metoprolol are finished some hours after the intake of the tablets.

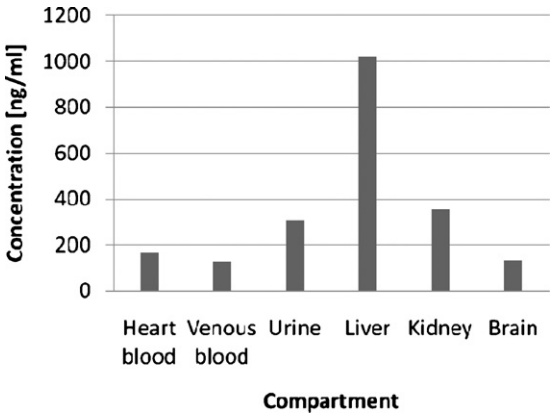

Fig. 2. Distribution of metoprolol after oral dosage and a short time between application and measurement—mean of three cases.

In urine samples the elimination of metoprolol can be observed. The elimination of metoprolol and its metabolites is the dominant process at this time.

In three cases the highest concentration of metoprolol was found in the liver. Probably metoprolol was taken shortly before the persons were killed in an accident, died after an embolism or after myocardial infarct. The main part of the last dose was not completely metabolized and distributed yet. Metoprolol measured in brain and in kidney tissue probably came from the dosage before the last one or the effect of a slow release formulation was observed. The drug concentration in blood was in the therapeutic range. The means of these three cases are shown in Fig. 2.

In the other three cases the highest concentration of metoprolol was found in the urine. The concentrations in the liver were clearly lower. This means the elimination process of the drug predominated at the time of death. The drug concentrations in blood were in the upper therapeutic range. See the means of these examples in Fig. 3.

With the distribution pattern of metoprolol it was possible to estimate the duration between drug intake and death.

3.2.2. Metoprolol concentrations after intravenous dosage

The dosage and the number of metoprolol injections were not given. In blood taken from within one of the heart's chambers (heart blood) the very high drug concentration of 1940 ng/ml was found. It was much higher than in venous blood (120 ng/ml) which was taken from the femoral vein. This means the drug was hardly distributed after injection and only a small part was absorbed into the organs. Metoprolol accumulated in the liver first. The second highest concentration (879 ng/g) was measured there. The drug was also absorbed in the kidneys (405 ng/g). The concentrations in the tissues were considerably higher than in peripheral venous blood (Fig. 4).

Table 3
Concentrations of metoprolol in different body liquids and tissues.

No.	Heart blood	Venous blood	Urine	Liver	Kidney	Brain
	Concentration of metoprolol [ng/ml or ng/g, respectively]					
Me-1	179	168	n.s.	1672	479	164
Me-2	n.s.	92	1912	81	151	127
Me-3	26	0	531	11	10	44
Me-4	230	196	407	1041	506	189
Me-5	53	43	427	185	83	70
Me-6	87	23	207	352	85	39
Me-7	1941	119	n.s.	871	405	159

n.s., no sample.

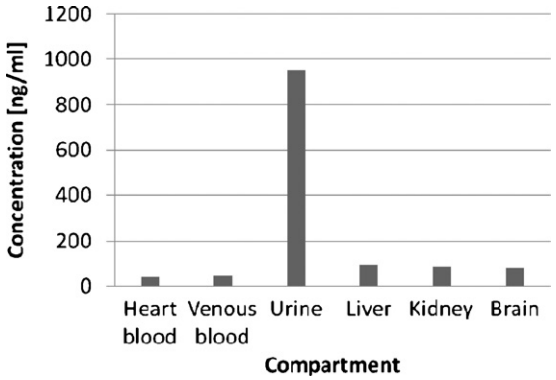

Fig. 3. Distribution of metoprolol after oral dosage and a long time between application and measurement—mean of three cases.

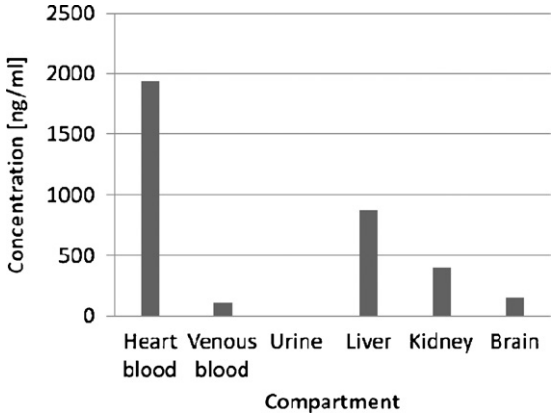

Fig. 4. Distribution of metoprolol after intravenous application.

3.2.3. Comparison of the metoprolol concentrations in the body liquids and organ tissues

With the exception of the intravenous application similar metoprolol concentrations were found in venous and in heart blood. In all cases the drug concentration in brain was similar to the concentration in venous blood. Metoprolol is uniformly distributed between heart blood, venous blood and brain independent of the time between application and measurement and independent of the size of dosage. An accumulation of metoprolol in the central nervous system can be excluded. To find out the cause of death it makes no difference to measure the metoprolol concentration in venous or in heart blood, with the exception of intravenous metoprolol applications.

The metoprolol concentrations in liver, the kidneys and urine showed high variations. The time between application of the drug and measurement of the concentration plays the crucial role for the distribution pattern of metoprolol in these compartments. The concentration in kidney tissue was on average 2–3 times higher than that in blood. Metoprolol blocks β-adrenergic receptors in the kidneys and in this manner reduces the plasma renin activity. On the other hand, there is no connection between drug concentration in the kidneys and in urine.

The β-adrenergic receptors in the heart are the main place of the effect of metoprolol but a measurement of the drug in heart tissue was not possible. Investigations of heart blood are not sufficient to describe the conditions in heart tissue. The metoprolol concentrations in both compartments can be very different.

In four cases the drug concentration in the liver was plainly higher than in the heart blood. A post mortem redistribution of drugs has often been described, especially from the organs to heart blood [6,30,31]. But in our case a redistribution of metoprolol from the liver to the heart blood against a concentration gradient is not possible.

3.3. Tramadol concentrations

The concentrations of tramadol in different body liquids and tissues are given in Table 4. After oral administration, tramadol is rapidly and almost completely absorbed. Sustained-release tablets release the active ingredient over a period of 12 h. Tramadol is rapidly distributed in the body. Tramadol is mainly metabolized by O- and N-demethylation and by conjugation reactions forming glucuronides and sulfates. Tramadol and its metabolites are mainly excreted via the kidneys. The mean elimination half-life is about 6 h. The O-demethylation of tramadol to M1, the main analgesic effective metabolite, is catalysed by cytochrome P450 (CYP) 2D6. The wide variability in the pharmacokinetic properties of tramadol can partly be ascribed to CYP polymorphism [8,9,32,33]. Tramadol is available as drops, capsules and sustained-release formulations for oral use, suppositories for rectal use and solution for intramuscular, intravenous and subcutaneous injection. In one case an intravenous application was given. The distribution pattern of this case was no different than the other cases with oral application. Therefore it was not discussed separately. The intravenous application had probably occurred a longer time before the death.

3.3.1. Tramadol concentrations after oral intoxication

The drug concentration in blood was higher than 5 μg/ml in three cases, thus an intoxication with tramadol was proved. The relatively uniform distribution in all compartments agrees with previous studies [10–12]. The very high concentration of tramadol in urine showed that the kidney functions a long time after taking the drug. The continuous taking of carbamazepin and the simultaneous intoxication with this anticonvulsant in case T2 could be the reason for the differences between the distribution patterns. Carbamazepin induces the enzyme CYP3A4 and the metabolism of tramadol is increased. For that reason less tramadol and more metabolites are excreted.

3.3.2. Tramadol concentrations after therapeutic dosage

The tramadol concentration in all compartments was similar. An accumulation was not observed with the exception of urine. No differences between drug distribution after oral and intravenous application were found. Bad blood circulation in the peripheral vessels could be the reason for low drug concentration in venous blood. The relatively uniform distribution in all organs, including the brain, agrees with distribution after intoxication described in this and in previous studies. The mean distribution pattern is given in Fig. 5. These results indicate that the distribution of tramadol is independent of the dose.

3.4. Midazolam concentrations

The concentrations of midazolam in different body liquids and tissues are given in Table 5. Compared to other benzodiazepines, midazolam exhibits a rapid onset of action and a fast hepatic elimination half-life (2–4 h). The clearance ranges between 400 and 600 ml/min [16–18,34,35].

3.4.1. Midazolam concentrations after intravenous therapeutic dosage

Midazolam was mostly proved after intravenous injections in connection with operations or emergencies. In all cases the dosage and the number of midazolam injections were unknown. In four cases the time between injection and death was documented. This duration plays an important role for the distribution of the drug. In two cases (Mi6 and Mi7) the highest midazolam concentration was

Table 4
Concentrations of tramadol in different body liquids and tissues.

No.	Heart blood	Venous blood	Urine	Liver	Kidney	Brain
	Concentration of tramadol [ng/ml or ng/g, respectively]					
T-1	602	449	n.s.	141	325	n.s.
T-2	8307	7248	31,627	22,714	8552	5544
T-3	25,892	5078	936,002	6515	10,194	14,078
T-4	249	69	1886	594	590	228
T-5	1196	896	n.s.	2022	896	709
T-6	12,789	8670	n.s.	21,522	9929	15,488
T-7	609	178	n.s.	432	678	479

n.s., no sample.

Table 5
Concentrations of midazolam in different body liquids and tissues.

No.	Heart blood	Venous blood	Urine	Liver	Kidney	Brain
	Concentration of midazolam [ng/ml or ng/g, respectively]					
Mi-1	n.s.	87	n.s.	6.9	7.3	300
Mi-2	164	22	n.s.	360	64	199
Mi-3	814	325	n.s.	1738	539	1062
Mi-4	n.s.	7,6	n.s.	20	13	326
Mi-5	n.s.	255	n.s.	235	1068	131
Mi-6	1359	708	n.s.	356	27	1229
Mi-7	2651	253	n.s.	99	0	1043
Mi-8	60	35	12	40	31	115
Mi-9	n.s.	10,1	n.s.	143	150	131
Mi-10	24	165	n.s.	45	55	195
Mi-11	265	68	66	111	80	2254
Mi-12	1224	326	n.s.	408	370	4419
Mi-13	783	461	n.s.	72	394	1138

n.s., no sample.

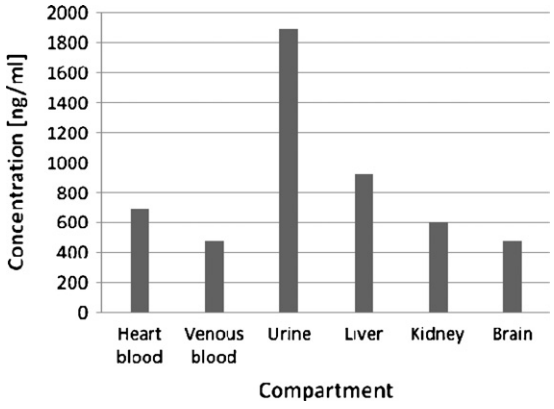

Fig. 5. Distribution pattern of tramadol after therapeutic application—mean of three cases.

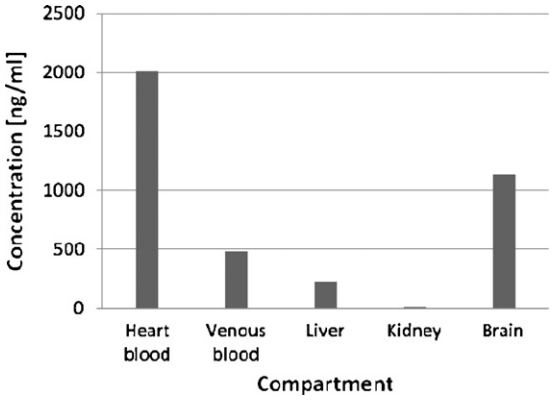

Fig. 6. Distribution of midazolam after intravenous dosage and a short time (approximately <30 min) between application and measurement—mean of two cases.

found in heart blood, this means midazolam was not completely distributed. In these cases the second highest drug concentration was found in the brain, see Fig. 6. Midazolam reached the brain a few minutes after injection. In six further cases the drug concentration in brain was the highest (heart blood samples of two cases missed). The similar distribution pattern of these six cases indicates similar duration between injection and death. In three times the duration was given with 30–45 min and in the three other cases this time can be estimated.

In one case the midazolam concentrations in the brain, liver, and kidney were similar and the duration between injection and death was 50 min. In three other cases (Mi2, Mi3, and Mi5) the drug concentrations in the excretory organs were higher than in the brain and blood, this means the distribution process took longer than 1 h and the elimination of the drug dominated, see Fig. 7.

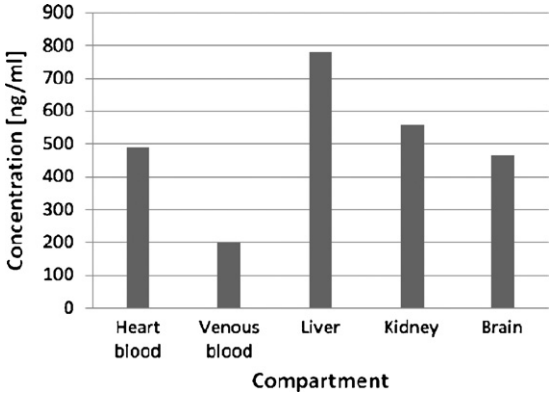

Fig. 7. Distribution of midazolam after intravenous dosage and a longer time (approximately 1 h) between application and measurement—mean of three cases.

3.4.2. Midazolam concentrations after oral therapeutic dosage

In the case with therapeutic oral drug application the highest concentration was found in the brain, the area of activity. The time between application and death by hanging was more than 3 h. The midazolam concentrations in the excretory organs were lower than in the brain and blood. This is surprising because the elimination of the drug should dominate some hours after application of the drug. Probably midazolam was metabolized in the liver but the metabolites were not covered in this study.

3.4.3. Comparison of the midazolam concentrations in the body liquids and organ tissues

Is there a significant difference between measurements in venous and in heart blood? This question is often discussed in legal medicine. Similar drug concentrations in cardiac and peripheral blood are expected in patients with oral application or continuous dosage. In these cases with bolus injections, a short time between injection and sampling or missing drug distribution in the body resulted in great differences between midazolam levels in venous and in heart blood. Both concentrations are not comparable and it is not possible to conclude from one to the other. In the examined cases no equilibrium between absorption, distribution and elimination could be observed. The distribution patterns changed quickly, depending on the duration between injection and death. In our study it was possible to show how midazolam was distributed from heart blood to brain and then to the excretory organs.

4. Conclusion

In this study it was possible to measure the distribution of metoprolol, tramadol or midazolam in human simultaneously and directly in several compartments for the first time. Investigations of autopsy material offered the opportunity of direct measurement of the drug distribution in human. It could be shown that the time between application and death exerted a crucial influence on the distribution of metoprolol after oral application and on distribution of midazolam after intravenous application. An additional result of the study was that the distribution of tramadol was independent of the dosage.

The determination of drugs in autopsy material deepens the knowledge of their pharmacokinetics.

References

[1] P. Benfield, S.P. Clissold, R.N. Brogden, Drugs 31 (1986) 376.
[2] M.J. Kendall, S.R. Maxwell, A. Sandberg, G. Westergren, Clin. Pharmacokinet. 21 (1991) 319.
[3] M. Junge, M. Tsokos, K. Puschel, Forensic Sci. Int. 113 (2000) 457.
[4] H. Kinoshita, T. Taniguchi, M. Nishiguchi, H. Ouchi, T. Minami, T. Utsumi, H. Motomura, T. Tsuda, T. Ohta, S. Aoki, M. Komeda, T. Kamamoto, A. Kubota, C. Fuke, T. Arao, T. Miyazaki, S. Hishida, Forensic Sci. Int. 133 (2003) 107.
[5] A. Mozayani, P. Singer, G. Jones, J. Anal. Toxicol. 19 (1995) 519.
[6] A.L. Pelissier-Alicot, J.M. Gaulier, C. Dupuis, M. Feuerstein, G. Leonetti, G. Lachatre, P. Marquet, Int. J. Legal Med. 120 (2006) 226.
[7] C.D. Riker, R.K. Wright, W. Matusiak, B.E. de Tuscan, J. Forensic Sci. 32 (1987) 1447.
[8] P. Dayer, L. Collart, J. Desmeules, Drugs 47 (Suppl. 1) (1994) 3.
[9] L.J. Scott, C.M. Perry, Drugs 60 (2000) 139.
[10] D.K. De, J. Cordonnier, W. Jacobs, V. Coucke, P. Schepens, P.G. Jorens, Forensic Sci. Int. 175 (2008) 79.
[11] B. Levine, V. Ramcharitar, J.E. Smialek, Forensic Sci. Int. 86 (1997) 43.
[12] T. Matthiesen, T. Wohrmann, T.P. Coogan, H. Uragg, Toxicol. Lett. 95 (1998) 63.
[13] K.A. Moore, S.J. Cina, R. Jones, D.M. Selby, B. Levine, M.L. Smith, Am. J. Forensic Med. Pathol. 20 (1999) 98.
[14] M.G. Ripple, J.P. Pestaner, B.S. Levine, J.E. Smialek, Am. J. Forensic Med. Pathol. 21 (2000) 370.
[15] B. Solarino, B. Riesselmann, C.T. Buschmann, M. Tsokos, Forensic Sci. Int. 194 (2010) e17–e19.
[16] R.J. Fragen, Clin. Ther. 19 (1997) 405.
[17] J.H. Kanto, Pharmacotherapy 5 (1985) 138.
[18] K.T. Olkkola, J. Ahonen, Handb. Exp. Pharmacol. (2008) 335.
[19] C.C. Young, R.C. Prielipp, Crit. Care Clin. 17 (2001) 843.
[20] G. Asselborn, M. Yegles, R. Wennig, Acta Clin. Belg. Suppl. (2002) 54.
[21] L.Y. Gorczynski, F.J. Melbye, J. Forensic Sci. 46 (2001) 916.
[22] C. Dupuis, J.M. Gaulier, A.L. Pelissier-Alicot, P. Marquet, G. Lachatre, J. Anal. Toxicol. 28 (2004) 674.
[23] M. Gergov, P. Nokua, E. Vuori, I. Ojanpera, Forensic Sci. Int. 186 (2009) 36.
[24] R.D. Johnson, R.J. Lewis, Forensic Sci. Int. 156 (2006) 106.
[25] L. Kristoffersen, E.L. Oiestad, M.S. Opdal, M. Krogh, E. Lundanes, A.S. Christophersen, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 850 (2007) 147.
[26] H. Umezawa, X.P. Lee, Y. Arima, C. Hasegawa, H. Izawa, T. Kumazawa, K. Sato, Biomed. Chromatogr. 22 (2008) 702.
[27] M.J. Kendall, J. Clin. Pharm. Ther. 14 (1989) 159.
[28] C.G. Regardh, G. Johnsson, Clin. Pharmacokinet. 5 (1980) 557.
[29] J. Wikstrand, B. Andersson, M.J. Kendall, H. Stanbrook, M. Klibaner, J. Cardiovasc. Pharmacol. 41 (2003) 151.
[30] R.E. Ferner, Br. J. Clin. Pharmacol. 66 (2008) 430.
[31] A.L. Pelissier-Alicot, J.M. Gaulier, P. Champsaur, P. Marquet, J. Anal. Toxicol. 27 (2003) 533.
[32] S. Grond, A. Sablotzki, Clin. Pharmacokinet. 43 (2004) 879.
[33] P.I. Hair, M.P. Curran, S.J. Keam, Drugs 66 (2006) 2017.
[34] U. Klotz, Anaesthesiol. Reanim. 14 (1989) 347.
[35] S.P. Spina, M.H. Ensom, Pharmacotherapy 27 (2007) 389.