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Distribution of metoprolol in human autopsy material

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Received June 2, 2010, accepted July 2, 2010

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Pharmazie 66: 115–118 (2011) *doi: 10.1691/ph.2011.0665*

In forensic medicine autopsy material is primarily 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 not being related to the cause of death. Liquid/liquid extraction and LC/MS/MS methods were used for the determination of drug concentrations. In seven cases metoprolol could be determined in different autopsy materials. In all cases the dosage of the drug was unknown. In cases with oral application probably the patients took a normal customary continuous dosage. Intoxication with metoprolol could be excluded in all cases. The concentrations of metoprolol in blood were all in the therapeutic range. The time between oral intake and death was unknown. Therefore and because of the low number of cases statistic calculations were not meaningful and an individual case study was necessary. In three cases the highest concentration of metoprolol was found in the liver. Probably, metoprolol was taken shortly before the person died. In the other cases the highest concentration of metoprolol was found in urine. This means the elimination process of the drug predominated at the time of death. In all cases the concentrations of metoprolol were similar in the compartments heart blood, venous blood and brain. In this study it was possible to measure the distribution of metoprolol in human directly in several compartments. Measurement of drug concentrations in human autopsy material deepen the knowledge of its pharmacokinetics.

1. Introduction

In forensic legal medicine autopsy material is primarily investigated to find out the cause of death. But the results of corresponding toxicology measurements often involve more information. With screening methods drugs were often detected not being related to the cause of death. Either the deceased had a continual therapeutic treatment or an unsuccessful urgent therapy. When a drug was identified in autopsy material, the sample was analyzed again after standard addition in this study.

In seven cases metoprolol, a ß1-selective adrenoceptor antagonist, could be proved and determined in different autopsy materials.

Metoprolol is widely used in the treatment of mild to moderate hypertension and angina pectoris. Blockade of the ß1-receptor reduces heart rate, myocardial contractility and cardiac output. Metoprolol reduces plasma renin activity. Dizziness, bradycardia and hypotension are observed as adverse reactions. 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 pKa = 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 Vd = 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 to 4 h (Benfield et al. 1986; Kendall 1989;1991; Regardh and Johnsson 1990; Wikstrand et al. 2003).

Numerous cases with metoprolol intoxication have been published (Junge et al. 2000; Kinoshita et al. 2003; Mozayani et al. 1995; Riker et al. 1987). Distribution of metoprolol and its *postmortem* redistribution in rabbits have been described. But animal experiments have a limited meaningfulness (Dupuis et al. 2004; Pelissier-Alicot et al. 2006).

In this study it was possible to measure the distribution of metoprolol in humans directly in several compartments using a LC/MS/MS screening method. Similar analytical methods have been reported (Dupuis et al. 2004; Johnson and Lewis 2006; Kristoffersen et al. 2007; Umezawa et al. 2008).

2. Investigations, results and discussion

2.1. Case reports

Seven cases with metoprolol in autopsy material were discovered. Short case reports are given in Table 1. In all cases the dosage of the drug was unknown. The measured concentrations in blood were in the therapeutic range or lower. In the six cases with oral application the patients probably had taken a normal customary continuous dose. Intoxication with metoprolol could be excluded. The case with intravenous application has to be considered separately. The concentrations of metoprolol in different body liquids and tissues are given in Table 2.

Table 1: Reported cases

No	Age (years)	Weight (kg)	Sex	Cause of death	Medication	Application	Time[1] (min)
1	70	96	female	craniocerebral trauma (accident)	continuous	oral	w.g.
2	57	76	female	intoxication with insuline	continuous	oral	w.g.
3	58	w.g.	female	intoxication with ethanol, doxylamine and temazepam	continuous	oral	w.g.
4	81	70	female	embolism	continuous	oral	w.g.
5	75	81	male	intestinal bleeding to death	continuous	oral	w.g.
6	57	w.g.	male	myocardial infarct	continuous	oral	w.g.
7	48	96	male	heart failure	emergency	intravenous	30

time[1] . . . time between application and death
w.g. . . . without giving

2.2. Metoprolol concentrations after oral therapeutic dosage

In six of the seven cases the metoprolol concentrations in blood were below 250 ng/ml. One can assume that in these cases 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 other body components. The liver metabolizes metoprolol, only a part of the active drug emerges from the liver to the scirculatory system. Shortly after oral intake, the drug concentration in the liver is high. Later, a steady state of absorption, distribution, and elimination of the drug is reached. Aabsorption and distribution of metoprolol are completed a few hours after intake of the tablets. Then, metoprolol can be detected in urine samples, as elimination of metoprolol and its metabolites is the dominant process at this time (Kendall et al. 1991).

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 or died following embolism or myocardial infarction. The main part of the last dose was not yet completely distributed and metabolized. Metoprolol measured in brain and in kidney tissue resulted either from a former dose or from a slow release formulation. The drug concentration in blood was in the therapeutic range. An example is shown in Fig. 1.

In the other three cases the highest concentration of metoprolol was found in urine. The concentrations in the liver were clearly lower. This means that the elimination process of the drug predominated at the time of death. The drug concentrations in blood were in the upper therapeutic range. See an example in Fig. 2.

Table 2: Concentrations of metoprolol (ng/ml es ug/g) in different body fluids and tissues

No	Heart blood	Venous blood	Urine	Liver	Kidney	Brain
1	179	168	n.s.	1672	479	164
2	n.s.	92	1912	81	151	127
3	26	< 5	531	11	10	44
4	230	196	407	1041	506	189
5	53	43	427	185	83	70
6	87	23	207	352	85	39
7	1941	119	n.s.	871	405	159

n.s. no sample

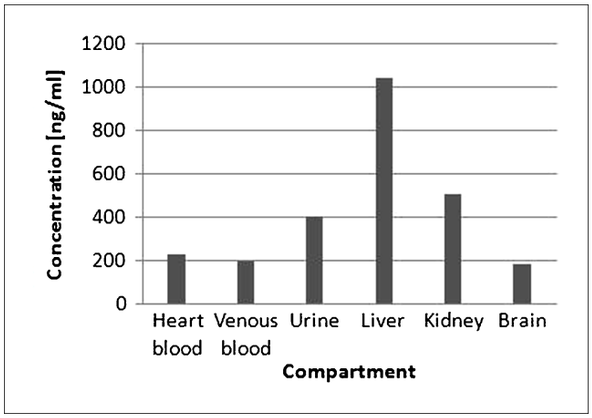

Fig. 1: Distribution of metoprolol after oral dosage and a short time between application and measurement

2.3. Metoprolol concentrations after intravenous dosage

The dosage and the number of metoprolol injections were not given. In case of heart failure up to three injections of 5 mg metoprolol are commonly given. In blood taken from within one of the heartïs 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. Only a small part was absorbed by the organs. Metoprolol was accumulated in the liver at first. The second highest concentration (879 ng/g) was measured there. The drug was also absorbed from the kidney (405 ng/g). The concentrations in the tissues were considerably higher than in venous blood (Fig. 3).

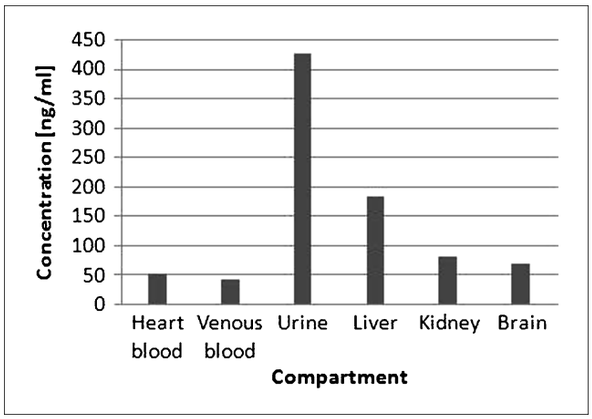

Fig. 2: Distribution of metoprolol after oral dosage and a long time between application and measurement

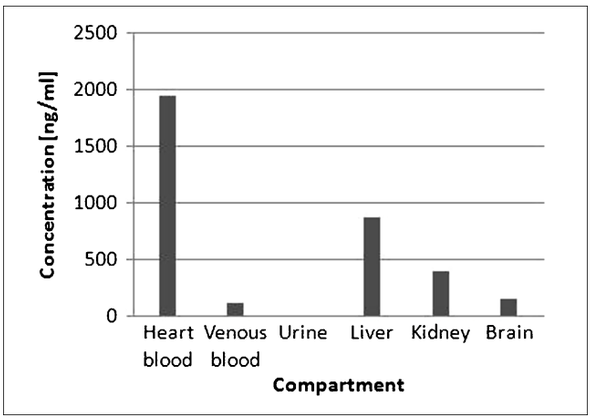

Fig. 3: Distribution of metoprolol after intravenous application

A *post mortem* redistribution of drugs has often been described, especially from the organs to heart blood (Ferner 2008; Johnson and Lewis 2006). But in our case a redistribution of metoprolol from the liver to the heart blood against a concentration gradient is not possible.

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

With the exception of the intravenous application similar metoprolol concentrations were found in venous blood 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 regardless of the time between application and measurement and regardless of the dose. An accumulation of metoprolol in the central nervous system could 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, kidney 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 in blood. Metoprolol blocks ß-adrenergic receptors in kidney and reduces the plasma renin activity in this manner. On the other hand there is no connection between drug concentration in kidney and in urine.
The beta adrenergic receptors in the heart are the main target 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 (Dupuis et al. 2004; Johnson and Lewis 2006; Kinoshita et al. 2003).
In four cases the drug concentration in the liver was plainly higher than in the heart blood. A post mortem redistribution of metoprolol from the liver to the heart could be excluded (Ferner 2008; Pelissier-Alicot et al. 2006).

3. Experimental

3.1. Chemicals

Metoprolol was purchased from Sigma (St. Louis, MO, USA). 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). Pure water (18 MΩ) was obtained using an ion exchange system RS 40 E, SG Ionenaustauscher (Barsbüttel, Germany).

3.2. Sample preparation and standard addition

A liquid-liquid extraction procedure was used for sample preparation. The samples were prepared twice: pure and spiked with 0.50 µg/g of metoprolol for quantification by standard addition procedure. 0.5 ml of heart blood, venous blood, and urine as well as 0.5 g of organ tissues (brain, liver, kidney), respectively, 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.

3.3. HPLC-System

The HPLC equipment consisted of a Dionex P680 HP-gradient pump and an autosampler Dionex ASI 100 T (Idstein, Germany) with a Chromeleon Chromatography Data System (Dionex Softron, Idstein, Germany). The chromatographic separation was performed on a Synergy 4 µ Polar-RP 80A, 150 mm x 2 mm (Phenomenex, Aschaffenburg, Germany) column with a Security Guard C18, 4 mm x 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 minutes 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 time of metoprolol was 3.8 min.

3.4. Mass spectrometer

The MS/MS system used was a Quattro micro (Micromass, Manchester, GB) equipped with an electrospray interface (ESI). For positive ionisation a capillary voltage of 3500 V and ion source temperature of 100 °C were applied. The desolvation gas flow (nitrogen) was 600 l/h at 300 °C.
Metoprolol was measured simultaneously with other analytes employing the multiple reaction monitoring mode (MRM) with the specific transition m/z = 268 → m/z = 116, a cone voltage of 24 V, a collision energy of 18 eV and the collision gas was argon.
The MassLynx Data System (Waters/Micromass, Manchester, GB) was applied for MS control and QuanLynx for peak area evaluation, regression analysis of standard curves, and calculation of concentrations.

3.5. Calculation

Standard addition procedure was used instead of an external calibration curve to solve the recovery and the matrix effect problems. A solution of the known concentration of metoprolol (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 metoprolol standard - were used to extrapolate and determine the initial concentration in the unknown sample.

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