Postmortem femoral blood concentrations of the antipsychotic drug risperidone and the active metabolite 9-hydroxyrisperidone were determined by an achiral LC–MS/MS method in 38 cases. The cause of death was classified as unrelated to risperidone in 30 cases, in which the sum of the concentration of the drug and metabolite ranged from below the limit of quantification to 0.058 mg/kg (median 0.0098 mg/kg). This concentration range, which largely corresponds to published in vivo plasma levels under therapy, may serve as a reference for judgment of postmortem cases involving risperidone. In one case, risperidone was judged to be a contributing factor to death, and the sum of concentrations was 0.29 mg/kg. This concentration is of the same order of magnitude as observed for plasma levels in clinical intoxication cases. For the remaining seven cases, the cause of death was unclear. The measurements observed here do not suggest that risperidone is subject to major postmortem redistribution.

Introduction

Risperidone is an antipsychotic drug widely used in the treatment of schizophrenia. The recommended daily dose is 2–6 mg orally, and administered in depot intramuscular form 25–50 mg once every 2 weeks (1). A racemate of the active metabolite, 9-hydroxyrisperidone, is available as a separate drug, paliperidone (2). Under ordinary therapy, the plasma level of risperidone averages 0.004–0.008 mg/L and that of 9-hydroxyrisperidone 0.010–0.025 mg/L (3). The higher level of the metabolite relates to a longer half-life (~21 h) than that of the parent compound (2.8 h) (4). 9-Hydroxyrisperidone possesses pharmacological activity equal to that of risperidone (1). The transformation to 9-hydroxyrisperidone is mediated by the polymorphic cytochrome P450 enzyme CYP2D6, which catalyzes the formation of the R-enantiomer of 9-hydroxyrisperidone, and CYP3A4 and CYP3A5, which play an important role in the formation of the S-enantiomer (5–7). In poor metabolizers with regard to CYP2D6, the concentration of the parent compound is increased, while that of the metabolite is decreased, so that the total concentration is largely the same as in extensive metabolizers. During therapy with risperidone in extensive metabolizers, the R-enantiomer of 9-hydroxyrisperidone attains higher concentrations than the S-enantiomer (7). In overdose cases, tachycardia, EKG-changes, confusion, neurological symptoms, drowsiness and seizures may occur (1). A fatality has been described with a blood concentration of ~1.8 mg/L risperidone (8).

In postmortem cases, drug blood levels may not be comparable with in vivo levels in plasma because of postmortem redistribution and differences between plasma and whole blood levels (9, 10). Druid and Holmgren and Reis et al. compiled postmortem drug levels and divided them into three categories: A: concentrations in cases ascribed to fatal intoxication with the drug as the sole cause of death; B: concentrations where the drug is considered a contributing cause of death; and C: concentrations observed when the drug is not considered to be directly related to the cause of death, e.g. suicide by hanging or shooting (11, 12). This categorization is primarily based on the circumstances and not on the observed drug concentrations. We and other authors have also provided postmortem reference values for various drugs, e.g. for amlodipine, olanzapine and quetiapine (13–15). The effects of postmortem redistribution and differences between plasma and whole blood concentrations are taken into account in these values. In this study, the postmortem concentration range for risperidone and the metabolite observed over recent years is presented, and the results are discussed relative to previously published findings. In addition, results of in vivo whole blood measurements are presented for comparison.

Experimental

Risperidone and (+)-9-hydroxyrisperidone from Janssen-Cilag (Birkerod, Denmark) were used as reference materials, and stock solutions of 1000 mg/L in methanol were prepared. Risperidone-D4 from Toronto Research Chemicals (North York, Canada) was used as an internal standard (IS), and a working solution of 0.20 mg/L in 0.1% (v/v) formic acid in methanol was prepared. Acetonitrile and methanol (LCMS Grade) were obtained from Fisher Scientific (Leicestershire, UK) and formic acid (98–100% GR for analysis) from Merck (Darmstadt, Germany). Purified water was obtained from a Millipore Synergy UV water purification system (Millipore A/S, Copenhagen, Denmark). 0.1% (v/v) formic acid in water and 0.1% (v/v) formic acid in methanol were prepared and stored at 4°C. Blank human whole blood (BB) anticoagulated with EDTA was obtained from the Blood Bank at Copenhagen University Hospital (Copenhagen, Denmark) and preserved by adding 1% sodium fluoride. The blood was screened for presence of common illegal and medicinal drugs, inclusive risperidone and 9-hydroxyrisperidone, and found to be negative. BB was used for the negative control, blank with IS, spiked calibrators and quality control (QC) samples. Authentic forensic samples of whole blood were preserved with 1% sodium fluoride. The calibration standards in whole blood (0, 0.002, 0.050 and 0.500 mg/kg) were prepared for each run by spiking blood with dilutions of the stock solution in 0.1% formic acid in methanol (20 μL per 0.200 g blood). QC samples in whole blood were prepared at two concentrations (0.020 and 0.200 mg/kg) and stored at −80°C. QC samples were analyzed in each series.

Analytical method

Concentrations of risperidone and total 9-hydroxyrisperidone enantiomers were determined by an achiral LC–MS/MS method. Whole blood was subjected to protein precipitation: 0.200 g
blood (determined by weighing) was spiked with 25 μL 0.20 mg/L IS-solution and mixed with 1200 μL cold 0.1% formic acid in methanol. To improve the precipitation, a disposable plastic stick was added to the solution before mixing for 3 min. The samples were cooled for 30 min at −20°C to improve phase separation and absolute recovery. The supernatant was removed to another glass tube after centrifugation at 5°C for 10 min at 1600g. The methanolic phase was evaporated under a stream of nitrogen at 40°C, and the remains were reconstituted in 200 μL solvent (1:4:5 acetonitrile:methanol:0.1% formic acid in water). The reconstituted solution was mixed thoroughly, and after another centrifugation for 10 min, the solution was moved to an amber vial. Five microliters of each extract was injected onto the LC–MS/MS system.

Chromatographic separation was performed using an Agilent 1100 series high-performance liquid chromatography (HPLC) system from Agilent Technologies (Walldorf, Germany) comprises a binary pump, an autosampler, and a thermostated column compartment fitted with a Zorbax SB C18 column (2.1 × 50 mm, i.d. 3.5 μm, Agilent Technologies) and a Gemini C18 guard column (4 × 2.0 mm, Phenomenex, Allerod, Denmark). The eluant was diverted to waste for the first 2.0 min after injection. An elution gradient consisting of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) (5% up to 90% B) separated the compounds within 11 min. The flow rate was 0.30 ml/min at a column temperature of 60°C. The retention time of risperidone and metabolite was 8.41 and 7.84 min, respectively, while relative retention times were 1.0 and 0.94 against the IS (Figure 1). The LC–MS/MS/MS method is used for determination of several basic drugs in whole blood, and is therefore not optimized only for these target analytes. The HPLC run time could therefore be reduced if required. A tandem mass spectrometer, Quattro micro from Waters (Manchester, UK), was coupled to the HPLC system. Data were acquired in the positive ionization mode with an electrospray source (ESI+). Multiple reaction monitoring analysis was used for data collection—two traces per target compound. For risperidone: 411→191(Q) and 411→82 with a cone voltage of 48 V and collision energy at 31 and 63 eV, respectively, and for the metabolite: 427→207(Q); 427→110 with a cone voltage of 48 V and collision energy at 27 and 49 eV, respectively. The trace for the IS was 415→195 with a cone voltage of 48 V and collision energy at 29 eV. The source and desolvation temperatures were 120 and 300°C, respectively. Argon was used as the collision gas at 0.041 mbar. The absolute recoveries of risperidone and metabolite were 88 and 85%, respectively. There was a linear range from 0.002 to 1.0 mg/kg for both target compounds. LOD was at 0.0002 mg/kg and LOQ was 0.002 mg/kg for risperidone and metabolite, respectively. The imprecision (CV) of the QC samples was <10% at both levels, and the bias was <12% also for both levels and target compounds. The ion ratio was 5.74 ± 10% for risperidone and 2.06 ± 10% for the metabolite.

Results and Discussion

Cases

Over the years 2005–2012, risperidone and metabolite were measured in postmortem femoral blood in 38 cases. Details concerning dosing were not available. Autopsies were performed within 1–4 days after the bodies arrived at the department of forensic medicine; storage was at 5°C. Blood was drawn from the femoral vein at the beginning of the autopsy after the body was opened without ligation of the iliac vein. One case was classified as a category B case, where risperidone was considered a contributing cause of death. A 57-year-old woman with diagnosed schizophrenia was found dead in her apartment with an empty container of risperidone. At the autopsy, tablet fragments were found in the stomach. The postmortem blood concentrations of risperidone and total 9-hydroxyrisperidone were 0.18 and 0.11, respectively, with a sum of 0.29 mg/kg. Additionally, zopiclone was present at a toxic level, 0.46 mg/kg, and there was a blood alcohol concentration of 1.6 g/kg. It was judged as a combination poisoning of risperidone, zopiclone and ethanol. In 30 cases, the cause of death was classified as unrelated to risperidone (category C cases). These cases included 10 cases of natural death (cardiovascular and/or pulmonary diseases and cerebral disease), 13 cases of poisoning with psychoactive drugs and/or ethanol, 3 fire-related cases, 3 cases of suffocation and one hanging case. For the remaining seven cases, the cause of death was uncertain (U). Additionally, for comparison, cases from living subjects involved in traffic cases for the period 2005–2012 were included (N = 12, category D).

Risperidone plus metabolite concentrations

Risperidone plus total 9-hydroxyrisperidone concentrations recorded in the case scenarios, categories B–D, are displayed in Table 1, and the risperidone plus metabolite values reported in the literature are indicated in Table II (16–21). The concentration in the category B case, sum 0.29 mg/kg, is of the order of magnitude observed in clinical intoxication cases (16, 17). It is observed here that the risperidone concentration exceeds the metabolite concentration contrary to the average relations for the C cases suggesting acute ingestion.

The sum concentrations of the category C cases, which ranged from below LOQ to 0.058 mg/kg, were of the same order of magnitude as reported in vivo therapeutic plasma levels, 0.003–0.090 mg/L (3, 18–20). The plasma/blood distribution ratio has been reported to be on average 1.5, which would suggest that plasma levels are expected to be a half-time higher than blood values, other factors being equal (1). Roman et al. reported a small series of postmortem values corresponding to C cases, which ranged from 0.0023 to 0.021 mg/kg (sum) and so tended to be slightly lower than our values (21). The concentrations of the category D cases, 0.003–0.117 mg/kg, tended to be in the same range. For the postmortem cases with unclear cause of death, the sum concentrations ranged from 0.005 to 0.13 with a median of 0.011 mg/kg.

Thus, for risperidone and its metabolite, the present measurements as well as literature values do not suggest the presence of postmortem redistribution with increase of postmortem blood values. Actually, in a recent study by Saar et al., a decrease of 9-hydroxyrisperidone up to 43% was observed under repeated sampling in the postmortem period (22). This is in opposition to the majority of antipsychotic drugs that tend to exhibit increases of postmortem values (22, 23). Thus, it may be difficult to assess whether a death related to use of these antipsychotics has occurred as an adverse event at a therapeutic level or as a result of
Figure 1. Ion chromatogram (quantitative trace, qualitative trace and IS trace) of (a) blank whole blood, (b) standard at 0.002 mg/kg blood and (c) case with 0.014 mg/kg risperidone and 0.015 mg/kg total 9-hydroxyrisperidone in postmortem blood.
poisoning due to an overdose (24, 25). For some drugs, evaluation of the drug/metabolite ratio may give an indication, but otherwise it is important to relate observed concentrations to relevant post-mortem reference ranges, as well as to the case circumstances.

Conclusion

In the present study, postmortem reference values for risperidone plus total 9-hydroxyrisperidone concentrations in femoral blood were compiled. It was concluded that the reference values for cases presumed to be unrelated to risperidone toxicity were of about the same magnitude as referenced in vivo therapeutic plasma values, suggesting that risperidone is not likely to be subject to postmortem concentration increases. In one combined poisoning case, the risperidone plus metabolite concentration was of the same order of magnitude as reported in clinical intoxication cases.

References


