Sensitivity Analysis of Blonanserin, a Novel Antipsychotic Agent, in Human Plasma by Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry

Tadashi OGAWA,*†1 Hideki HATTORI,*1 Rina KANEKO,*2 Kenjiro ITO,*1 Masayo IWAI,*1 Yoko MIZUTANI,*1 Tetsuya ARINOBU,*3 Akira ISHI,*2 Osamu SUZUKI,*4 and Hiroshi SENO*1

*1 Department of Legal Medicine, Aichi Medical University School of Medicine, Nagakute, Aichi 480-1195, Japan
*2 Department of Legal Medicine and Bioethics, Nagoya University Graduate School of Medicine, 65 Tsuruma, Showa, Nagoya 466-8550, Japan
*3 Department of Chemistry, Aichi Medical University School of Medicine, Nagakute, Aichi 480-1195, Japan
*4 Department of Legal Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi, Hamamatsu 431-3192, Japan

A rapid and sensitive method for analysis of blonanserin in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry is presented. After pretreatment of a plasma sample by solid-phase extraction, blonanserin was analyzed by the system with a C18 column. This method gave satisfactory recovery rates, reproducibility, and good linearity of calibration curve in the range of 0.01 – 10.0 ng/mL for quality control samples spiked with blonanserin. The detection limit was as low as 1 pg/mL. This method seems very useful in forensic and clinical toxicology and pharmacokinetic studies.

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Introduction

Blonanserin (2-(4-ethyl-1-piperazinyl)-4-(4-fluorophenyl)-5,6,7,8,9,10-hexahydropyridazine; Fig. 1) is a novel antipsychotic agent, having dopamine D2 and serotonin 5-HT2A receptor antagonist properties.1–6 It is one of the second-generation antipsychotic agents, together with risperidone and olanzapine, it is effective in the treatment of both positive and negative symptoms of schizophrenia without extra-pyramidal symptoms, but has original properties of affinity higher for the dopamine D2 receptor than for the serotonin 5-HT2A receptor.1–4,6,7 On the other hand, blonanserin is much less potent in adrenergic-α1, histamine H1 and muscarinic M1 antagonist activities.6 Such a pharmacological profile shows that blonanserin is more specific to the dopamine D2 and serotonin 5-HT2A receptors with fewer side effects; its excellent effects on schizophrenia have been reported in many reports.7–10 There is a possibility that this drug will gain popularity for treatment of schizophrenia throughout the world.

For analysis of blonanserin, we could find a report using high-performance liquid chromatography (HPLC) with fluorescence detection.11 As the second report,12 we have presented a method for analysis of blonanserin in human plasma by gas chromatography/mass spectrometry (GC/MS). The HPLC method11 could not give the final identification data, and the detection limit of the GC/MS method12 was about 0.25 ng/mL. In this brief report, we present a high-throughput and much more sensitive method for analysis of this drug in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) using AD-5332 (Fig. 1) as internal standard (IS).

Experimental

Chemicals

Blonanserin and AD-5332 (IS) were donated by Dainippon Sumitomo Pharmaceutical (Osaka, Japan). Acetonitrile and distilled water (DW) of the HPLC grade were purchased from Kanto Chemical (Tokyo, Japan). Other common chemicals used were of the highest purity commercially available.
Solid-phase extraction procedure

The solid-phase extraction of blonanserin and IS was performed essentially according to our previous report\textsuperscript{12} with minor modifications. A 1.0-mL volume of plasma containing or not containing blonanserin was mixed with 5 ng IS and 2.0 mL of 200 mM HCl, vortex-mixed for 1 min, and centrifuged at 1600\textgreek{g} for 5 min. For solid-phase extraction, the supernatant fraction was applied to an Oasis HLB cartridge (60 mg, 3 cc; Waters, Milford, MA) preconditioned with 3 mL methanol and 3 mL DW. Each cartridge was washed with 3 mL DW. The columns were dried under vacuum for 10 s. The analytes were eluted with 3 mL chloroform, and the eluate was evaporated to dryness under nitrogen stream at room temperature. The residue as described above was reconstituted in 25 \textmu L of MeOH and 75 \textmu L of 0.1\% formic acid and vortex-mixed for 1 min.

UPLC-MS/MS conditions

The liquid chromatography (LC) instrument used in combination with an MS/MS detector was a Waters Acquity UPLC system, including an Acquity UPLC binary pump and a sample manager (Waters). The column used for chromatographic separation was Acquity UPLC BEH C\textsubscript{18} (50 × 2.1 mm i.d., particle size 1.7 \textgreek{m}m; Waters). The column temperature was maintained at 40°\textgreek{C}, and the following gradient system was used with a mobile phase A (20 mM ammonium acetate aqueous solution) and mobile phase B (0.1\% formic acid in acetonitrile) delivered at 0.5 mL/min. The linear gradient program was: 70\% A/30\% B (0 min)–5\% A/95\% B (4 min). The final mobile phase was used as a wash solvent to avoid any carry-over from previous injections. The autosampler was maintained at 4°\textgreek{C} and the injection volume was 5 \textmu L. The total run time for each sample analysis was 6.0 min.

The MS/MS detection was made using peak areas in a positive ion mode on a tandem quadrupole mass spectrometer (Acquity QTD; Waters) equipped with an electrospray ionization (ESI) interface. Quantitation was performed using selected reaction monitoring (SRM) with the transitions of \textit{m/z} 368→297 for blonanserin and \textit{m/z} 396→297 for IS. The optimal MS parameters were: capillary voltage, 3.0 kV; cone voltage, 30 V; source temperature, 120°\textgreek{C}; desolvation temperature, 450°\textgreek{C}; desolvation and cone gas, nitrogen with flow rates of 800 and 50 L/h, respectively; collision gas, argon with a flow rate of 0.15 mL/min; the optimized collision energies for blonanserin and IS, 25 eV each. All data in the centroid mode were acquired and processed using a MassLynx NT 4.1 software with a QuanLynx program (Waters).

Human experiments

The chief scientist of this research project (healthy 60-year-old male, 65.0 kg) volunteered as the subject for the human experiments. He took a single oral dose of 10 mg blonanserin, and his blood was sampled 1.5 and 4 h after intake. Blank blood was also sampled just before the drug intake. Plasma samples were immediately prepared by centrifuging the blood samples and stored at –20°\textgreek{C} until analyses. For quality control samples, blank blood was collected from healthy volunteers, and plasma was separated by centrifugation and stored at –20°\textgreek{C} as described above.

Results and Discussion

Product ion mass spectra and selected reaction monitoring chromatograms

The UPLC-single stage mass spectra obtained from blonanserin and IS spiked into blank human plasma showed protonated molecular peaks at \textit{m/z} 368 and 396, respectively, which appeared as base peaks. Other peaks appearing in their spectra were very small and thus could be neglected. The product ion mass spectra obtained from the protonated molecular ions of the authentic blonanserin and IS are shown in Fig. 2. Base peaks at \textit{m/z} 297 appeared for both compounds. The peaks are probably formed by the cleavage in the middle of the piperazine rings of each compound, which gave the ions of the same mass number at \textit{m/z} 297. Thus, we used this ion for quantitation of blonanserin and IS.

Figure 3 shows SRM chromatograms for blonanserin and IS spiked into blank human plasma at the concentration of 10 pg/mL. Blonanserin and IS appeared at 1.75 and 2.10 min, respectively. There were no impurity peaks until 3.0 min of retention time.

Reliability of the method

The peak ratios of blonanserin to IS obtained from SRM chromatograms were plotted at ten different concentrations against the concentration of blonanserin in human plasma, and a linear relationship was observed for blonanserin in the range of 0.01 – 10 ng/mL. The equation and its correlation coefficient were: \textit{y} = 0.296\textit{x} – 0.001 and \textit{r}\textsuperscript{2} = 0.999. The limit of detection (LOD), defined as the concentration giving the signal-to-noise ratio of 3, was about 1 pg/mL with quality control samples of human plasma. The recovery rates of blonanserin spiked into blank human plasma at concentrations of 0.01 and 10.0 ng/mL were 93.7 ± 1.2 and 93.7 ± 1.1\% (\textit{n} = 5 each), respectively.

Table 1 shows accuracy and precision data of the present method. Accuracy values were 86.9 – 103\%; precision values were not greater than 5.5\%.
In the first report describing analysis of blonanserin in plasma by HPLC with fluorescence detection, the linearity was demonstrated in the range of 0.04 – 5 ng/mL, but HPLC without MS cannot give the final identification of analytes. In the second report for analysis of the drug in human plasma by GC/MS, the linear range of the calibration curve was 0.5 – 20 ng/mL, and the detection limit was about 0.25 ng/mL. The present UPLC-MS/MS method for blonanserin is more than 50 times as sensitive as the GC/MS method. Such ultra-high sensitivity obtained by combination of UPLC with MS(/MS) was demonstrated for scopolamine in hair and for a new cannabimimetic indole in a herbal product in forensic toxicological analysis. It is probably due to sharp peaks produced by UPLC plus low backgrounds produced by the tandem MS mode.

**Table 1** Accuracy and precision data of the method

<table>
<thead>
<tr>
<th>Measurement interval</th>
<th>Concentration added/ng mL⁻¹</th>
<th>Accuracy, %</th>
<th>Precision, CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-day</td>
<td>0.01</td>
<td>87.7</td>
<td>4.9</td>
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<tr>
<td></td>
<td>10.0</td>
<td>100</td>
<td>2.1</td>
</tr>
<tr>
<td>Inter-day</td>
<td>0.01</td>
<td>86.9</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>103</td>
<td>2.3</td>
</tr>
</tbody>
</table>

CV: coefficient of variation.
Each value was obtained by 5 determinations.

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**Fig. 3** Selected reaction monitoring (SRM) chromatograms for blonanserin and IS spiked into blank human plasma obtained by ultra-performance liquid chromatography-tandem mass spectrometry. The amounts of blonanserin and IS spiked into 1 mL plasma were 10 pg and 5 ng, respectively.

**Fig. 4** SRM chromatograms for blonanserin and IS extracted from plasma obtained from a volunteer 4 h after administration.

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**Fig. 4** SRM chromatograms for blonanserin and IS extracted from plasma obtained from a volunteer 4 h after administration.

**Actual analysis of blonanserin in human plasma after single oral dose**

A 60-year-old volunteer ingested 10 mg blonanserin (therapeutic dose), and his blood was sampled 1.5 and 4 h after ingestion. For blood taken just before ingestion, no peak appeared at 1.75 min in an SRM chromatogram at m/z 297 (data not shown). Intense peaks appeared in both SRM chromatograms obtained 1.5 and 4 h after the intake (Fig. 4). The concentrations of blonanserin in plasma were 0.45 and 1.01 ng/mL after 1.5 and 4 h, respectively.

**Conclusions**

To our knowledge, this is the first report describing LC-MS/MS analysis of blonanserin. Various psychotropic drugs, such as minor and major tranquilizers and antidepressants, are of great importance in forensic toxicology, because such drugs can be abused, cause death, or influence the cause of death. Because blonanserin is classified as a major tranquilizer (a second-generation antipsychotic), cases of accidental death or suicide involving blonanserin will appear in the near future. Furthermore, the ultra-high sensitivity of the method allows the measurements of very low concentrations of blonanserin in biomedical matrices. The present proposed method seems very useful in forensic and clinical toxicological analyses, especially when only small amounts of blood specimens are obtainable, and also in pharmacokinetic studies of this drug.
Acknowledgements

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References