Characterization of Lacosamide Metabolites by UHPLC–ESI–HRMS Method

Maciej Gawlik, Robert Skibiński* and Łukasz Komsta
Department of Medicinal Chemistry, Medical University of Lublin, Jacezkiewicza 4, 20-090 Lublin, Poland

Received: 09 January 2019; accepted: 30 March 2019

In this study, the in vitro phase I metabolism of lacosamide was characterized with the use of ultra-high-performance liquid chromatography combined with high-resolution mass spectrometry (quadrupole time-of-flight). The use of two metabolism simulation techniques (photocatalysis and human liver microsomes) allowed the characterization of a polar metabolite of parent compound, not yet described. The experiment with the participation of HLM gave the ability to describe the full liver metabolic pathway of lacosamide. It has been proven that this molecule undergoes deacetylation, demethylation, and during liver tissue metabolism. Photocatalysis with the use of a TiO2 catalyst was proved to be a complementary technique in mimicking in vitro drug metabolism.

Keywords: HLM, LC–MS, metabolism, TiO2, photocatalysis

Introduction

Drugs, as molecules of non-natural chemical character for the human, are subjected to processes of absorption, distribution, metabolism, and excretion (known as ADME), regardless of the route of their administration [1–3]. Metabolism is one of the most important elements of this sequence. Its detailed knowledge provides a lot of valuable information, which may allow, for instance, the improvement of the absorption of the molecule at the site of administration. It is estimated that almost 10% of marketed drugs are distributed in the prodrug form, which are chemically converted into an active form after dosage. Prodrugs are usually less active or inactive, but their specific physicochemical properties help to improve the bioavailability of compounds [4–6]. On the other hand, undesirable toxic products may also appear in the phase I of metabolism of the therapeutic substance. Therefore, understanding the exact metabolism of drugs has a huge impact on the production of new drugs. It is an expensive process, and the occurrence of unforeseeable toxic or reactive products of biotransformation is definitely not recommended.

The liver is an organ responsible for the metabolism of many drugs [7, 8]. In order to minimize the costs and accelerate the research procedure, human liver microsomes (HLMs) were successfully used to simulate these processes. During the incubation of an analyzed drug with HLMs, it is possible to obtain information about metabolic pathways and to achieve biotransformation products that can be subjected to more detailed investigation.

A further attempt to reduce costs and human inputs, with simultaneous simplification of the procedure, resulted in the development of a method for simulating metabolism with the use of photocatalysis. Irradiation of a drug substance solution in the presence of photocatalyst molecules (TiO2) with a specific wavelength causes the oxidation in a manner similar to the processes of biotransformation [9]. The use of the combination of both biological and photocatalytic techniques can be considered as a complementary tool in drug metabolism investigations [10–14].

Epilepsy is one of the most common human neurological disorders, and it is connected with brain dysfunction, which reveals recurrent epileptic seizures caused by the abnormal activity of brain neurons. The disease has important, negative neurobiological, psychological, and cognitive consequences [15, 16]. The difficulty of the treatment with still high mortality has become the reason for searching for new and convenient pharmacological therapies [17].

Lacosamide, (R)-2-acetamido-N-benzyl-3-methoxypromipamide, is an anti-epileptic drug (AED) mainly used as a support in therapy of focal onset seizures, as well as neuropathic pain disease [18]. In 2017, European Medicines Agency allowed its application in the partial-onset seizures treatment of patients aged 4 years and older [19, 20]. Lacosamide stands out from other AEDs due to the novel mechanism of its action. Drug molecule selectively enhances slow inactivation of voltage-gated sodium channels. Unlike others, it does not affect the fast inactivation route. This action results in the stabilization of hyperexcitable neuronal membranes, which prevents seizures [21–24]. Lacosamide undergoes hepatic metabolism, mainly through the CYP2C19 enzymes; however, the exact biotransformation pathway has not been described yet. It was found that the main metabolite is O-desmethyl lacosamide with no anticonvulsant activity. Other metabolites, especially from polar fraction, have not been characterized so far [25, 26].

The main goal of this study was to characterize the lacosamide phase I metabolites in human liver microsomes and to compare the obtained results with photocatalysis transformation processes based on the use of TiO2. The use of both techniques allowed the establishment of complete hepatic metabolism pathway of lacosamide. Moreover, a polar metabolite of lacosamide, which is not so far described, was structurally characterized.

Materials and Methods

Chemicals and Reagents. Pharmaceutical formulation of lacosamide (Vimpat) was obtained in a local pharmacy, and water (LC–MS Ultra grade), β-nicotinamide adenine dinucleotide 2'-phosphate (NADPH)-reduced tetrasodium salt

DOI: 10.1556/1326.2019.00591
© 2019 The Author(s)

First published online: 19 May 2019
hydrate, human liver microsomes, sodium phosphate monobasic monohydrate salt, sodium phosphate dibasic anhydrous salt, tungsten (VI) oxide nanopowder 100 nm particle size, and titanium (IV) oxide nanopowder 21 nm particle size (Aeroxide® 25) were obtained from Sigma-Aldrich (St. Louis, USA). Acetonitrile (hypergrade for LC-MS) was purchased from Merck (Darmstadt, Germany), and 98% formic acid (LC-MS grade) was obtained from Fluka (Taufkirchen, Germany).

Ultra-High-Performance Liquid Chromatography– Electrospray Ionization–High-Resolution Mass Spectrometry (UHPLC–ESI–HRMS) Analysis. LC–MS analysis was performed with the use of a high-resolution (HR) Agilent Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS G6520B system with a dual electrospray ionization (DESI) source and an Infinity 1290 Ultra-High-Performance Liquid Chromatography–MS system consisting of a binary pump G4220A, a FC/ALS thermostat G1330B, an autosampler G4226A, a diode-array detector (DAD) G4212A, a thermostatted column compartment (TCC) G1316C module (Agilent Technologies, Santa Clara, USA), and a Kinetex C18 (2.1 mm × 50 mm, dp = 1.7 μm) column with a C18 precolumn guard (Phenomenex, Torrance, USA). A mixture of ultrapure water with addition of 0.1% solution of formic acid (A) and acetonitrile (B) was used as a mobile phase. The gradient elution was carried out at constant flow 0.3 mL min⁻¹ from 5% B (95% A) to 35% B 0–9 min, 2 min post time was performed to return to initial conditions. The injection volume was 4 μL and the column temperature was maintained at 35 °C. MassHunter workstation software in version B.08.00 was used for the control of the system, data acquisition, qualitative and quantitative analysis.

The optimization of the instrument conditions started from the proper tuning of Q-TOF detector in a positive mode with the use of Agilent ESI-L tuning mix in the extended dynamic range (2 GHz). The following instrument settings were applied: gas temp.: 250 °C, drying gas: 10 L/min, nebulizer pressure: 40 psig, capillary voltage: 1500 V, fragmentor voltage: 125 V, skimmer voltage: 65 V, octopole 1 RF voltage: 750 V.

Data acquisition was performed in centroid mode with the use of the TOF (MS) and auto tandem mass spectroscopy (MS/MS) mode. The spectral parameters for both modes were a mass range of 60–950 m/z and an acquisition rate of 1.5 spectra per s. To ensure accuracy in masses measurements, a reference mass correction was used, and masses 121.050873 and 922.009798 were used as lock masses.

Sample Preparation. The active substance was isolated from the tablets. For the pharmaceutical formulation of lacosamide, the average mass tablet was determined, and an equivalent of 1 mM lacosamide was transferred to a 10-mL volumetric flask containing 5 mL of water. After 5-min ultrasonic sweeping, the sample was diluted up to volume with water, and then centrifuged at 13,500 rpm for 5 min (at room temperature). This stock solution after adequate dilution was used for both HLM and photocatalytic experiments.

In Vitro Metabolism Study in HLM. Phase I metabolism reactions of lacosamide were studied in vitro with the use of human liver microsome fraction. Incubation system consisted of 0.05 mM substrate, 50 mM phosphate buffer (pH 7.4),

Table 1 Q-TOF accurate mass elemental composition and MS/MS fragmentation and of the analyzed substances

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Name</th>
<th>Retention time (min)</th>
<th>Measured mass [m/z]</th>
<th>Theoretical mass [m/z]</th>
<th>Mass error [ppm]</th>
<th>Molecular formula [M + H]⁺</th>
<th>MS/MS fragm [m/z]</th>
<th>Fragmentation ion formula [M + H]⁺</th>
<th>Observed in HLM TiO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lacosamide</td>
<td>3.85</td>
<td>251.1382</td>
<td>251.1390</td>
<td>−3.18</td>
<td>C₁₁H₁₇N₂O₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M1</td>
<td>1.98</td>
<td>209.1289</td>
<td>209.1284</td>
<td>2.39</td>
<td>C₁₁H₁₇N₂O₂</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>M2</td>
<td>0.60</td>
<td>146.0806</td>
<td>146.0811</td>
<td>−3.42</td>
<td>C₁₁H₁₇N₂O₂</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>M3</td>
<td>2.58</td>
<td>237.1200</td>
<td>237.1233</td>
<td>−13.92</td>
<td>C₁₁H₁₇N₂O₂</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>5</td>
<td>M4</td>
<td>2.10</td>
<td>267.1313</td>
<td>267.1339</td>
<td>−9.73</td>
<td>C₁₁H₁₇N₂O₂</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>6</td>
<td>M5</td>
<td>2.50</td>
<td>267.1337</td>
<td>267.1339</td>
<td>−0.74</td>
<td>C₁₁H₁₇N₂O₂</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>7</td>
<td>M6</td>
<td>3.20</td>
<td>267.1340</td>
<td>267.1339</td>
<td>0.37</td>
<td>C₁₁H₁₇N₂O₂</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>
and 0.5 mg/mL microsomes. HLM samples were pre-incubated at 37 °C for 2 min, and then, the metabolic reaction was initiated by addition of 10 μL NADPH (20 mM). Total volume of reaction suspension was 200 μL. The reaction was terminated after 30, 60, 90, 180, 270, and 360 min of incubation with 200 μL ice-cold acetonitrile–methanol mixture (1:1). Next, the precipitated samples were centrifuged at 13,500 rpm for 10 min at 4 °C, and the supernatants (50 μL) were transferred into autosampler vials for LC–ESI–HRMS analysis. The negative control samples were prepared as described above without addition of NADPH solution.

**Photocatalytic Experiments.** The photocatalytic reactions were performed in water solution with 0.025 mM lacosamide

![Figure 1. MS/MS spectrum and fragmentation pathway of lacosamide](image)
and 0.1 mg/mL TiO\textsubscript{2}. For all experiments, the formed suspensions were transferred into 3.5-mL quartz caped cells (l = 1 cm) and stirred in the dark at 500 rpm (microstirrer Cimarec, Telemodul, Thermo Electron LED GmbH, Germany) for 15 min to achieve adsorption–desorption equilibrium. Next, the reaction cells were mounted horizontally in an Atlas SUNTEST CPS+ photostability chamber with a D65 filter (Linsengericht, Germany and irradiated simultaneously with stirring. The irradiance was set to 750 W m\textsuperscript{2}, which corresponds to energy dose of 2700 kJ/m\textsuperscript{2} h. The temperature in the chamber was controlled and kept below 35 °C. Aliquots (100 μL) were collected at proper intervals and time ranges 0–16 min. Suspensions were then centrifuged in the Eppendorf vials (MPW-375 centrifuge, MPW Med. Instruments, Poland) at 13,500 rpm for 5 min, 50 μL aliquots were collected, and UHPLC–ESI–HRMS analysis was performed.

Results and Discussion

Identification of Lacosamide Metabolites by Mass Spectrometry. In order to identify the structure of the lacosamide metabolites, HRMS analysis was performed with the use of auto MS/MS mode. Based on the obtained high-resolution spectra, 6 metabolites of lacosamide were found during this study (Table 1). Registered MS/MS spectra and fragmentation patterns of lacosamide and the detected products (M1–M6) were presented in Figures 1–7.

The spectra of M1–M3 metabolites were generated by the use of HLM, as well as with the photocatalytic method. The spectra of M4–M6 metabolites were generated from the photocatalytic experiment due to the inadequate quality of the spectrum obtained on microsomes.

As shown on the MS/MS spectrum of lacosamide (Figure 1), the fragmentation occurs in two ways due to the gradual degradation of the aliphatic chain with the formation of an ion with m/z 177.1008 (C\textsubscript{10}H\textsubscript{13}N\textsubscript{2}O). The fragment with m/z 108.0808 (C\textsubscript{7}H\textsubscript{10}N), which corresponds to phenylmethanaminium ion, is clearly marked. Fragments with m/z 144.0651 (C\textsubscript{6}H\textsubscript{10}NO\textsubscript{3}), 116.0704 (C\textsubscript{5}H\textsubscript{10}NO\textsubscript{2}), and 74.0609 (C\textsubscript{3}H\textsubscript{8}NO) indicates the further gradual decomposition of the aliphatic chain.

Metabolite M1 (m/z 209.1289, C\textsubscript{11}H\textsubscript{17}N\textsubscript{2}O\textsubscript{2}) was identified as a descarbonyl-lacosamide (Figure 2). Fragmentation gradually leads to N-methylthalamamide ion (m/z 74.0596, C\textsubscript{3}H\textsubscript{8}NO) appearance, the most abundant structure in the spectrum. The
ion with \( m/z \) 91.0546 (C_{7}H_{7}) identified as a phenylmethylium ion is also clearly marked.

The M2 metabolite (\( m/z \) 146.0806, C_{6}H_{12}NO_{3}) was marked as a product of aliphatic chain detachment from the phenylmethanamine structure and specified as a N-(1-methoxy-3-oxopropan-2-yl)acetamide. Removal of the methoxy moiety results in the formation of an N-(1-oxopropan-2-yl)ethanaminium ion (\( m/z \) 116.0707, C_{5}H_{10}NO_{2}). The cleavage of 2 oxygen atoms leads in turn to the formation of an ion with \( m/z \) 84.0815 (C_{5}H_{10}N) – the most abundant fragment in the spectrum which corresponds to N-ethenylpro-1-en-2-aminium ion (Figure 3).

Desmethyl lacosamide (M3) with \( m/z \) 237.1200 (C_{12}H_{17}N_{2}O_{3}) was found to be the third most abundant hepatic metabolite in our experiment (Figure 4). The ions with \( m/z \) 108.0807 (C_{5}H_{12}N) and \( m/z \) 91.0542 (C_{3}H_{7}) are the two most visible fragments in the spectrum, and they come from the gradual removal of the aliphatic chain from parent molecule. The most characteristic fragment with \( m/z \) 130.0497 (C_{3}H_{7}NO_{3}) was found as a (2-acetamido-3-hydroxypropylidene)oxidanium ion.

The M4–M6 metabolites were found to be the hydroxylated derivatives of lacosamide. M4 (\( m/z \) 267.1313, C_{13}H_{18}N_{2}O_{4}) was identified as 4-hydroxy-lacosamide (Figure 5). Characteristic fragment with \( m/z \) 107.0494 (C_{3}H_{7}O), which belong to the most abundant ion in fragmentation pattern, verifies the creation of (4-methyldienecyclohexa-2,5-dien-1-ylidene)oxidanium ion as a product of aliphatic chain detachment. Additional fragments with \( m/z \) 144.0656 (C_{11}H_{18}N_{2}O_{4}) and 116.0704 (C_{5}H_{10}NO_{2}) indicate further degradation of the aliphatic part of the compound. M5 (\( m/z \) 267.1337, C_{13}H_{18}N_{2}O_{4}) was characterized as a 3-hydroxy-lacosamide. The presence of ions with \( m/z \) 144.0647 (C_{11}H_{18}NO_{3}) and \( m/z \) 124.0756 (C_{7}H_{10}NO) with further creation of fragment with \( m/z \) 107.0493 (C_{3}H_{7}O) from (3-hydroxyphenyl)methylion indicates the preferred route for fragmentation of the compound. M6 (\( m/z \) 267.1340, C_{13}H_{18}N_{2}O_{4}) was identified as 2-hydroxy-lacosamide (Figure 7). Fragmentation in the case of this compound leads to gradual aliphatic chain degradation with creation ions with \( m/z \) 235.1076 (C_{12}H_{17}N_{2}O_{3}) and 225.1239 (C_{11}H_{17}N_{2}O_{3}). The most abundant in the spectrum is the fragment with \( m/z \) 107.0492 (C_{3}H_{7}O) identified as a (6-methyldienecyclohexa-2,4-dien-1-ylidene)oxidanium ion, which come from the second in turn \( m/z \) 124.0758 (C_{7}H_{10}NO). It should be noticed that M4–M6 metabolites were registered only on metabolic profiles.

![Figure 3. MS/MS spectrum and fragmentation pathway of M2](image-url)
obtained by photocatalytic approach. The use of this method allowed the verification of the presence of at least some trace amounts of these metabolites in given retention times on HLM profiles.

**Evaluation of the Hepatic Lacosamide Metabolites Formation.** In this study, only 3 metabolites from the hepatic pathway were obtained as a result of lacosamide incubation with the HLM fraction during 360 min (Figure 8). First of all, descarbonyl derivative (M1) was found to be a most abundant hepatic metabolite of lacosamide. The increase of this product in time is almost linear and grows up to the end of incubation. M2 metabolite, determined as a N-(1-methoxy-3-oxopropan-2-yl)acetamide appeared 60 min after the start of the experiment and reached higher values than M3 after 100 min. Formation kinetics of this metabolite is very similar to the main metabolite; however, its abundance is about twice lower. Desmethyl lacosamide (M3) appeared shortly after M1, reached maximum in the range of 90−270 min, and after that, its abundance apparently decreased. Its formation differs significantly from other metabolites (M1, M2) in both kinetic and quantitative terms, and it should be treated as a minor derivative in this case.

**Pathways of Lacosamide Metabolism.** Obtained results showed that lacosamide undergoes the three main metabolic pathways involving hepatocellular tissues. Deacetylation was found to be a preferred reaction type and facilitated the creation of M1 metabolite identified as a descarbonyl-lacosamide. The second most abundant derivative of lacosamide was characterized as a N-(1-methoxy-3-oxopropan-2-yl)acetamide (M2). It is a new transformation product, not described yet in the literature, of lacosamide, and its formation has been additionally verified by a photocatalytic process with the use of TiO2. Thus, its identification allowed the determination of the polar metabolic fraction of the test compound. The M3 product, which is a result of demethylation of lacosamide molecule, was found to be the third abundant metabolite. The Obtained results allow us to conclude that the formation of M4–M6 metabolites takes place with the participation of extrahepatic mechanisms, and their occurrence in urine may indicate renal metabolic pathway. Additionally, the hydroxylated and simultaneously desmethylated derivatives of lacosamide, described earlier in the literature [26], seem to indicate the complicity of the extrahepatic pathway in their origin. The proposed phase I
hepatic metabolism pathway of lacosamide is shown in Figure 9.

Conclusion

In this study complete phase I hepatic metabolism of lacosamide was proposed. With the use of UHPLC-ESI-HRMS a new, not described yet in the literature, polar metabolite of lacosamide-N-(1-methoxy-3-oxopropan-2-yl)acetamide was identified. This product was characterized as one of the two main metabolites and therefore, the necessity of its determination, especially in the case of the pharmacokinetic research or monitored therapies, should be considered.

Additionally, the results obtained with HLM were compared with those of photocatalytic techniques, which also made it possible to achieve the same transformation product. The combination of both techniques has found application in drug metabolism studies, and the photocatalytic method proved to be complementary to biological and enabled the rapid generation of metabolites, also unobtainable on the liver microsomes.

References

Figure 6. MS/MS spectrum and fragmentation pathway of M5
Figure 7. MS/MS spectrum and fragmentation pathway of M6
Figure 8. Evolution profiles of hepatic metabolites of lacosamide

Figure 9. The proposed hepatic metabolic pathway of lacosamide