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Novel tandem hydrogen migrations across five chemical bonds for 1,4-dihydropyridine using electrospray ionization FT-ICR mass spectrometry

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ABSTRACT

The electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR MS) was applied to investigate the characteristic fragmentation patterns of 1, 4-dihydropyridines using collision-induced dissociation (CID) method in positive ion mode. The intra-molecular tandem hydrogen atom migrations were observed with gas phase Na^+/H^+ exchange by crossing five chemical bonds for 1,4-dihydropyridines. The possible rearrangement mechanisms were proposed for the first time, and the key structure of product ions were confirmed by high resolution tandem mass spectrometry and in solution hydrogen/deuterium labeling. The novel tandem hydrogen migration could be considered as a general fragmentation pattern for 1,4-dihydropyridine drugs because of their intrinsic chemical structures.

1. Introduction

1,4-Dihydropyridines as calcium channel blockers have proved to be one of the most important chemical classes for the treatment of hypertension, cardiac dysrhythmias and angina [1–5]. Since the introduction of first drug nifedipine with proved antihypertensive activity through blocking of a voltage-gated calcium channel, dihydropyridine-type drugs have been developed as the largest group of calcium antagonists containing more than 20 drugs in pharmaceutical market [6]. Indeed, the 1,4-dihydropyridine nucleus is the chemical basis of nicotinamide adenine dinucleotide as coenzyme in cell metabolism [7–9]. Because of its importance for drug discovery, 1,4-dihydropyridine nucleus as the excellent pharmacophore has been recognized as the class of "privileged structures". The general chemical structures of 1,4-dihydropyridines are shown in Scheme 1. Owing to the wide use of these drugs in the therapy of hypertension, great many of analytical methods based on high performance liquid chromatography-mass spectrometry (LC–MS) have been successfully developed with very high selectivity and sensitivity for the identification and quantification of calcium channel antagonists in biological fluids [10–18]. The fragmentation patterns of 1,4-dihydropyridines using electrospray ionization mass spectrometry (ESI–MS) are very important not only for the determination of target drugs and their related metabolites, but also for the selection of parent/product ion pairs for multiresidue LC–MS quantifications. It has been reported that most 1,4-dihydropyridines were mainly fragmented through the cleavage of side ester bonds from 3- or 5-positions by loss of one molecule of alcohol or alkylene [19–21]. Recently, hydride abstraction of 1,4-dihydropyridines has been observed as new gas phase chemistry using ESI–MS [22]. However, the basic fragmentation mechanisms of 1,4-dihydropyridines are not addressed so far [23].

In present work, the fragmentation behaviors of 1,4-dihydropyridines were investigated in detail using high resolution electrospray ionization Fourier transform ion cyclotron resonance (FT-ICR) tandem mass spectrometry in combination with stable isotope labeling technique. Surprisingly, it is found that the hydrogen atom attached to nitrogen atom in 1,4-dihydropyridine ring could intra-molecularly migrate to carbonyl oxygen atom in side chain of ester by crossing five chemical bonds. Furthermore, an aromatic pyridine ring could be formed after tandem hydrogen atom migrations. The possible rearrangement mechanisms of hydrogen migrations were proposed and further confirmed by the solution deuterium/hydrogen labeling.

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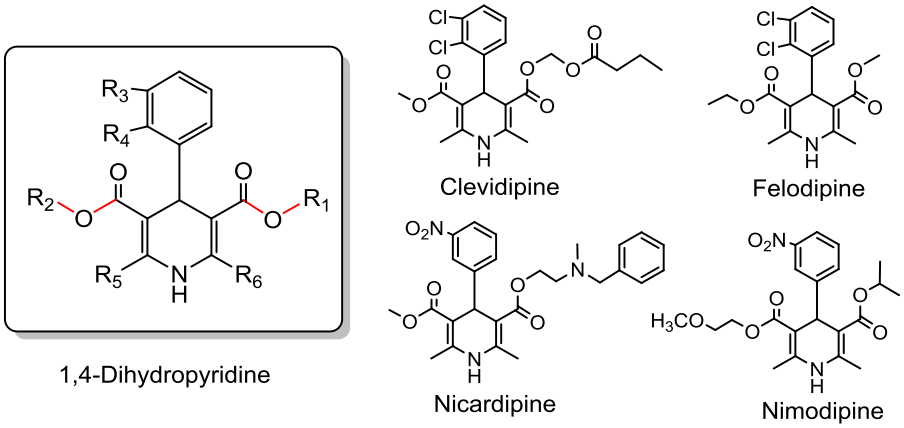

Scheme 1. The general chemical structures of 1,4-dihydropyridines and four drugs studied in present study.

2. Experimentals

2.1. Chemicals

Clevidipine, felodipine, nicardipine, and nimodipine were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. The chemical structures of the compounds studied are shown in Scheme 1. HPLC-grade methanol was purchased from Tedia (Fairfield, OH, USA). Deionized water (18.2 MΩ) used in all experiments was prepared from a Milli-Q water purification system (Millipore, Bedford, MA, USA). D_2O and CH_3OD (99.9 at% D) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA).

2.2. Mass spectrometry conditions

Mass spectrometry analyses were performed on Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Bremen, Germany) with 7 T magnet (Magnex, UK) and electrospray ionization source (Apollo II, Bruker Daltonics, Bremen, Germany) in positive ion mode. The conditions employed for ESI source were set as follow: a drying gas temperature of 180 °C, a nebulizing gas pressure of 1.0 L/min, with 4.0 kV on the atmospheric side of the glass capillary and 3.5 kV on the atmospheric chamber end cap shield. The capillary exit voltage was kept at 36 V to avoid capillary-skimmer dissociation in the ESI interface. All ion excitations were performed in broad band mode (frequency sweep radial ion excitation). The base pressure in the ICR vacuum chamber was 4.0 e-11 mbar and in the quadrupole region 6.0 e-6 mbar. High pressure in the quadrupole region was necessary to colinearize the ions in the radialplane and decelerate the radial components of the ion kinetic energy. For CID experiments, argon was used as the collision gas and the collision energy was set to 15 eV. The precursor ions such as $[M+H]^+$ and $[M+Na]^+$ were isolated and analyzed by tandem mass spectrometry (MS/MS). 1,4-Dihydropyridines (10 ppm) in methanol/water (v/v, 1:1) were injected directly to the ionization source by a microliter pump at a flow rate of 2.0 μL/min. For deuterium labeling experiment, D_2O/CH_3OD (v/v, 1:1) was used as testing solvent resulting in the complete exchange of labile protons in the compounds according to the previous protocol [24].

$ESI–MS^3$ analysis was performed by using a Bruker Esquire 3000plus ion trap mass spectrometer (Bruker Daltonic Inc., Billerica, MA, USA) equipped with a gas nebulizer probe capable of analyzing ions up to *m/z* 6000 in positive ion mode. Clevidipine was dissolved in methanol and infused continuously into the ESI chamber at a flow rate of 4 μL/min by a model 74900 syringe pump. Nitrogen was used as the drying gas at a flow rate of 4 L/min and the capillary temperature was 200 °C. The targeted precursor ions were selected using an isolation width of 1.0–1.5 mass to charge units. The scan range was generally from *m/z* 50–800. Five scans were averaged for each spectrum.

3. Results and discussion

Four 1,4-dihydropyridines including clevidipine, felodipine, nicardipine, and nimodipine with different side chain groups were intensively analyzed by using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer (ESI-FT-ICR MS) in positive ion mode. The fragmentation data are summarized in Table 1. As shown in Fig. 1 (A) and Fig. S1 (Supporting information), the sodium adducts $[M+Na]^+$ ions, such as *m/z* 478.0787 for clevidipine (Theoretical mass, *m/z* 478.0800, relative error 2.7 ppm), *m/z* 406.0586 for felodipine (Theoretical mass, *m/z* 406.0589, relative error 0.7 ppm), and *m/z* 441.1627 for nimodipine (Theoretical mass, *m/z* 441.1638, relative error 2.5 ppm), are observed as base peak under our mass spectrometry conditions. As shown in Fig. 1 (A), several peaks including *m/z* 478.0787, *m/z* 479.0831, *m/z* 480.0770, *m/z* 481.0804, and *m/z* 482.0748 with different relative intensities were detected for $[M+Na]^+$ ion of clevidipine by high resolution ESI-FT-ICR MS. The characteristic isotope patterns observed are perfectly in agreement with the simulated spectrum of compound containing two chloride atoms. Furthermore, $[M+K]^+$ ion of clevidipine at *m/z* 494.0536 (Theoretical mass, *m/z* 494.0534, relative error 0.4 ppm) was also observed with the same isotopic patterns. In order to study the fragmentation pathways of 1,4-dihydropyridine, the sodium adduct ions of clevidipine as representative compound was firstly isolated by the quadrupole rod of FT-ICR MS and subjected to tandem mass spectrometry for further collision-induced dissociation in gas phase.

The ESI–MS/MS spectrum of $[M+Na]^+$ ion of clevidipine is shown in Fig. 1 (B) and the possible fragmentation pathways are summarized in Scheme 2. The precursor $[M+Na]^+$ ion could cleave the ester C—O bond to generate two abundant product ions at *m/z* 360.0165 and *m/z* 338.0346 under collision in gas phase. It is interesting to find that the isotope patterns of two product ions are very similar to that of precursor ion with the characteristic ^{35}Cl and ^{37}Cl isotope ratios, indicating that two chloride atoms do not lose during fragmentation. In addition, the mass difference determined with high resolution between two product ions is 21.9818, which is nearly same to the mass difference (21.9819) between Na^+ (Theoretical mass, *m/z* 22.9892) and H^+ (Theoretical mass, *m/z* 1.0073) ions. Therefore, it could deduce that two product ions might have the same chemical structures with different ions attached. As shown in Scheme 2, the exact mass determined for **4a** and