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Metabolism studies of ifenprodil, a potent GluN2B receptor antagonist

Evamaria Falck, Frank Begrow, Eugen Verspohl, Bernhard Wünsch*

Institut für Pharmazeutische und Medizinische Chemie, Westfälische Wilhelms-Universität, Münster, Germany

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ABSTRACT

The NMDA receptor antagonist ifenprodil is an important lead structure for developing new GluN2B selective NMDA receptor antagonists. Ifenprodil itself has a high affinity to the GluN2B subunit but a poor selectivity for the NMDA receptor. This aspect and the fast biotransformation are the major drawbacks of ifenprodil. In order to optimize the development of new and more selective GluN2B (NMDA) receptor antagonists, the identification of the main metabolic pathways of ifenprodil is necessary.

Herein the *in vitro* and *in vivo* phase I and phase II metabolites of ifenprodil were generated and analyzed *via* LC–MS^n experiments. *In vitro* experiments were carried out with rat liver microsomes and various co-factors to generate phase I and phase II metabolites. The application of ifenprodil to a rat and the analysis of its urine led to the identification of diverse formed *in vivo* metabolites. The phenol represents the metabolically most labile structural element since glucuronide **7** and **8** appeared as main metabolites.

1. Introduction

Ifenprodil, originally developed as a vasodilating agent due to its antagonistic activity at α_1 receptors, attracted more attention when its high affinity to the GluN2B-subunit of the *N*-methyl-D-aspartate (NMDA) receptor was revealed. This subunit is able to serve as one of four subunits forming the heterotetrameric NMDA receptor associated ion channel. The subunits of the NMDA receptor belong to three groups: GluN1 (eight splice variants a–h), GluN2 (four types A–D) and GluN3 (two types A, B). Each receptor consists of at least one GluN1 and one GluN2 subunit [1]. The NMDA receptor is located in the brain and spinal cord [2]. Its concentration in different regions of the central nervous system (CNS) and composition change during the development of the brain during lifetime period. The NMDA receptor represents an interesting target for drugs useful for the treatment of epilepsy [3]. It is also involved in the development of neurodegenerative diseases such as schizophrenia, Alzheimer's or Parkinson's disease, making it an promising target for the development of innovative drugs [2]. Damage of cells leads to an increased release of (*S*)-glutamate which results in overactivation of glutamate receptors (*e.g.* AMPA, kainate and NMDA receptors) inducing further neuronal cell death [1]. This so-called excitotoxicity process reinforces the neurodegenerative effects. Compounds regulating the activity of NMDA receptors can be useful as neuroprotective agents.

Interaction of ifenprodil with the NMDA receptor reduces the opening state of the ion channel and inhibits the influx of Ca^{2+} ions. With respect to this mechanism ifenprodil leads to neuroprotective, anticonvulsant and analgesic effects. The binding site of ifenprodil was first supposed at the amino terminal domain (ATD) of the GluN2B subunit [4]. Later it could be found at the surface between the GluN1 and the GluN2B subunits [5]. According to the first developed ligand it has been termed "ifenprodil binding site" and can cross-talk with different other binding sites of the receptor [4]. The NMDA affinity of ifenprodil is very high (IC_{50} = 13.3 nM, K_i = 10 nM), but its selectivity is rather poor [6,7]. The interaction of ifenprodil with other receptors in the CNS (α_1, 5-HT, σ_1, σ_2 receptor), leads to undesired side effects, *e.g.* impaired motor function and reduced blood pressure. Nevertheless, ifenprodil serves as an important lead structure for the rational design of novel GluN2B selective antagonists bearing the potential of becoming drugs for life-threatening CNS diseases [2].

It has been reported that the bioavailability of Ifenprodil is rather low [8]. The maximum plasma level in humans was found after ~30 min [9]. However the biotransformation of ifenprodil and the structure of its metabolites have not been described so far. Therefore, identification of the metabolically labile positions of ifenprodil

Abbreviations: BSA, bovine serum albumin; CNS, central nervous system; COMT, catechol-*O*-methyl transferase; EIC, extracted ion current; NADPH, nicotinamide adenine dinucleotide phosphate; NMDA, *N*-methyl-D-aspartate; PAPS, adenosine-3′-phosphate-5′-phosphosulfate lithium salt hydrate; SAM, *S*-(5′-adenosyl)-L-methionine iodide; TIC, total ion current; UDPGA, uridine 5′-diphosphoglucuronic acid trisodium salt.

* Corresponding author at: Institut für Pharmazeutische und Medizinische Chemie, Corrensstr. 48, 48149 Münster, Germany. Tel.: +49 251 83 33311; fax: +49 251 83 32144.
E-mail address: wuensch@uni-muenster.de (B. Wünsch).

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might contribute to the development of metabolically more stable drugs interacting with the ifenprodil binding site. Herein we report on our studies of phase I and phase II metabolism of ifenprodil *in vitro* and *in vivo*.

2. Materials and methods

2.1. Chemicals and materials

Ifenprodil tartrate salt, *S*-(5′-adenosyl)-L-methionine iodide (SAM), uridine-5′-diphosphoglucuronic acid trisodium salt (UDPGA), adenosine-3′-phosphate-5′-phosphosulfate lithium salt hydrate (PAPS) and Coomassie Brilliant Blue were purchased from Sigma–Aldrich (Munich, Germany). NADPH sodium salt was obtained from Carl Roth (Karlsruhe, Germany). Acetonitrile in HPLC grade was delivered by VWR (Darmstadt, Germany). Formic acid was purchased from Merck KGAA (Darmstadt, Germany). Purified water for HPLC analysis and sample dilution was generated by a Milli-Q Advantage Ultrapure Water System, Millipore (Billerica, MA, USA). All other chemicals were obtained from different suppliers in analytical grade. SPE cartridges with octadecyl stationary phase were obtained from J. T. Baker® (Philipsburg, MT, USA). The metabolism cage (serial number 3700M071) was manufactured by Tecniplast Metabolics (Hohenpreissenberg, Germany).

2.2. Animals

For isolating liver microsomes and for *in vivo* metabolism studies of ifenprodil Wistar rats weighing 200–320 g from a local strain were used (Charles River Laboratories, Sulzfeld, Germany). Rats were allowed food and water *ad libitum*. The experiment was approved by the German animal welfare committee (AZ.84-02.04.2013.A148).

2.3. Preparation of rat liver microsomes

Livers of Wistar rats both sexes were obtained from Charles River Laboratories (Sulzfeld, Germany). Livers (50 g) were washed with cold 1.15% (m/v) potassium chloride solution and homogenized in an Elvehjem-Potter homogenizer with equal volume of cold phosphate buffer (pH 7.4, 0.1 M) containing sodium EDTA (0.5 mM). The resulting suspension was diluted with 140 mL sodium phosphate buffer and centrifuged for 20 min at 4 °C at 9000 × *g*. The supernatant was centrifuged at 40,000 × *g* for 60 min. The resulting microsome pellet was dissolved in sodium phosphate buffer (0.1 M, pH 7.4) and stored at −80 °C prior to use.

Protein concentration was determined according to BRADFORD and STOSCHECK [10,11]. A solution of Coomassie Brilliant Blue G 250 (5 mg) in ethanol (2.5 mL) was diluted with water (10 mL) and

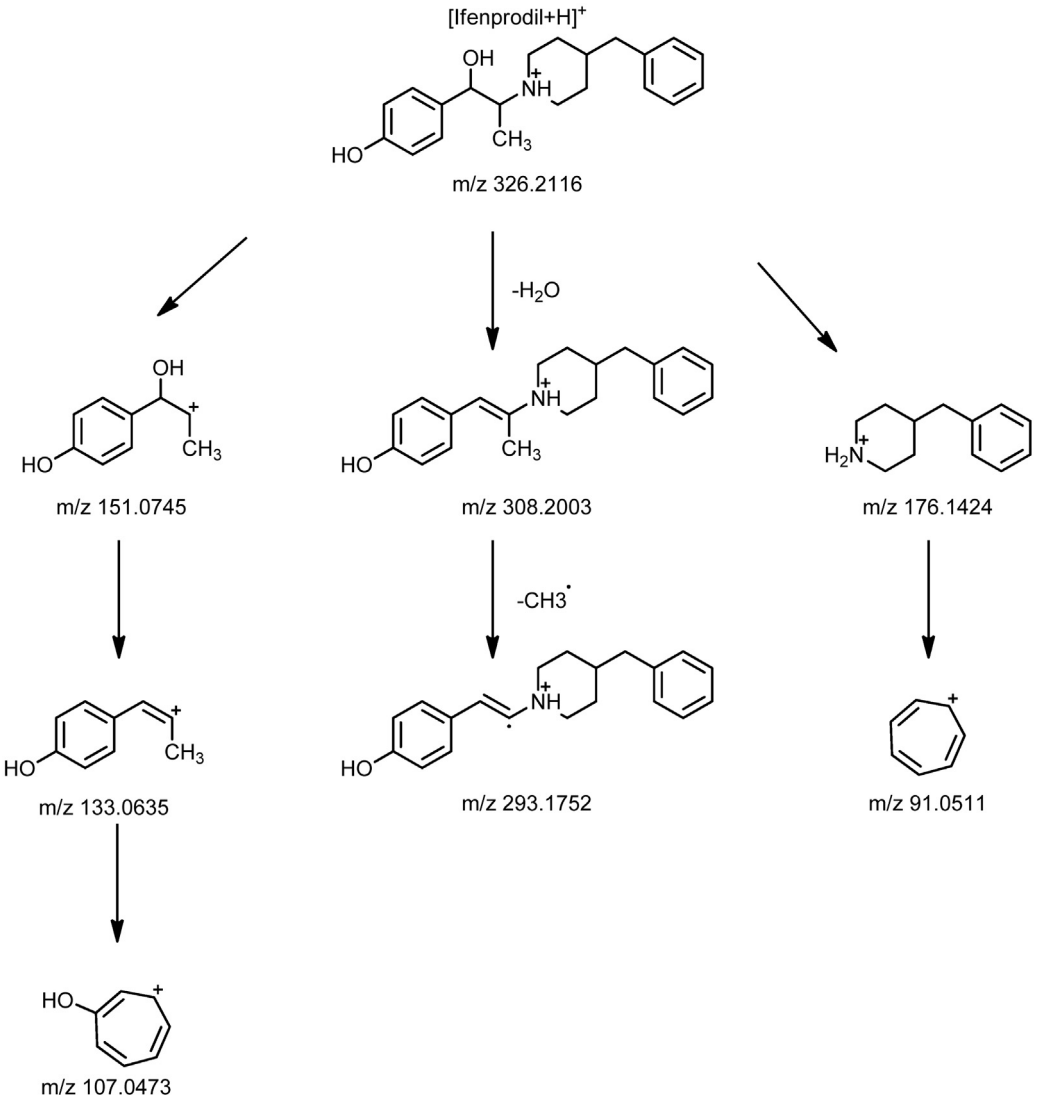

Fig. 1. Fragmentation of the parent compound ifenprodil. All *m*/*z* values are shown as observed in MS experiments (HPLC–ESI-IonTrap).