Forensic Science International 243 (2014) 112–116

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Pregabalin serum levels in apprehended drivers☆

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ARTICLE INFO

Article history:
Available online 6 July 2014

Keywords:
Pregabalin
Drugs of abuse
DUID
Driving

ABSTRACT

Pregabalin is a medicinal drug used mainly for the treatment of epilepsy and neuropathic pain. It has been shown to possess an abuse potential and in recent years some reports of illegal use have been published. In order to further evaluate the extent and nature of pregabalin abuse, serum pregabalin levels of drivers apprehended for driving under the influence of drugs (DUID) in Finland in 2012 were assessed. The samples were analysed by an LC–MS/MS system and the results were evaluated in relation to the typical therapeutic range of pregabalin as well as the age and gender of the driver.

Pregabalin was detected in 206 samples in the study period. The median (range) serum concentration was 6.2 (0.68–111.6) mg/L. In nearly 50% of the cases the serum concentration was above the typical therapeutic range. In most of the cases the driver had also taken other drugs besides pregabalin, the mean number of concomitantly taken drugs being four.

Our data indicate that pregabalin is being used at high doses, probably for recreational purposes. The vast majority of the drivers positive for pregabalin in our study material had used pregabalin as a part of a spectrum of psycho-active drugs and thus qualified as probable drug abusers. In these cases pregabalin probably contributed to their driving impairment but to what extent remained unclear in this study.

1. Introduction

Pregabalin is a psycho-active medicinal drug that exhibits analgesic, anticonvulsant, anxiolytic, and antidepressant effects. It was launched in 2004 in the EU and in the USA, and is marketed with the trade name Lyrica (Pfizer, NY, USA). In the EU, pregabalin is approved for the treatment of epilepsy, neuropathic pain, and generalized anxiety disorder [1]. In the United States, pregabalin is approved for the treatment of neuropathic pain associated with diabetic neuropathy, post herpetic neuralgia, epilepsy (partial onset seizures), and fibromyalgia [2]. Pregabalin was the first drug to be approved for the treatment of fibromyalgia by the U.S. Food and Drug Administration (FDA) [3].

Pregabalin (*S*-(+)-3 isobutyl-GABA) (Fig. 1) is a structural analogue of γ-amino butyric acid (GABA), but is not functionally related to it. Its mechanism of action is not completely understood but it binds to the α2δ subunit of neuronal voltage-dependent calcium channels resulting in reduced influx of calcium ions into the neuron, thus decreasing the release of neurotransmitters [4].

The typical therapeutic dose of pregabalin is 150–600 mg per day divided into 2–3 doses. Plasma levels at typical therapeutic doses of pregabalin in the treatment of neuropathic pain are in the range of 2.0–8.0 mg/L [5,6]. Steady state plasma concentrations of up to 14.2 mg/L were reported when 600 mg per day were administered in a clinical setting for patients with partial seizures [7]. The half-life of the drug is 5–11 h. Steady state plasma concentration is achieved in 24–48 h with twice daily dosing. The side effects of pregabalin include e.g. dizziness, somnolence, loss of consciousness, confusion, mental impairment, and blurred vision [1,8]. Pregabalin exhibits very high bioavailability [9]. It is mainly excreted in urine as unchanged drug and there is practically no

☆ This paper is part of the special issue entitled "The 51st Annual Meeting of the International Association of Forensic Toxicologists (TIAFT)". September 2–3, 2013, Funchal, Medeira, Portugal. Guest edited by Professor Helena Teixeira, Professor Duarte Nuno Vieira and Professor Francisco Corte Real.

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http://dx.doi.org/10.1016/j.forsciint.2014.06.030
0379-0738/

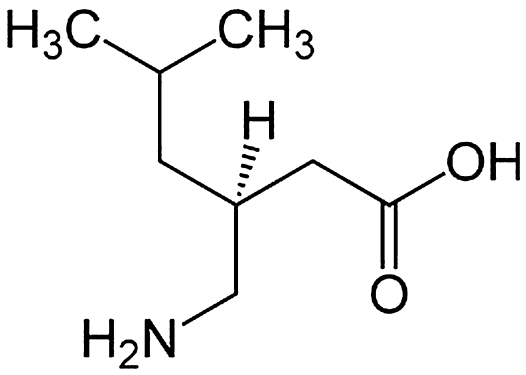

Fig. 1. The structure of pregabalin, (*S*)-3-(aminomethyl)-5-methylhexanoic acid.

metabolism observed in humans [9] resulting in a linear pharmacokinetic profile, lack of genetic variability in the drug pharmacokinetics and lack of drug interactions. These characteristics simplify the clinical use of pregabalin [10].

In the U.S. Controlled Substances Act, pregabalin is categorised as a Schedule V drug, the category which represents the lowest potential for abuse [11].

Although pregabalin was first believed to possess negligible abuse potential, an increasing number of reports about the abuse and addictive potential of pregabalin have been published in recent years [12–16]. In Finland, in 2010, there were 15 fatalities attributed to pregabalin: most of these deaths were known drug abusers [17]. This put pregabalin in 11th place among the most prevalent causes of fatal intoxications in Finland. Altogether pregabalin was detected in 67 fatalities cases in that year in Finland [17].

The effects of pregabalin on driving performance have been studied in a driving simulation experiment where the drug was shown to cause mild effects on some components of driving performance but no serious CNS side effects were found [18]. However, to the best of our knowledge, there are no published data on the blood concentrations or the prevalence of pregabalin in drivers suspected of driving under the influence of drugs (DUID). The aim of this study was to assess the serum levels of pregabalin in DUID cases. In our study, pregabalin serum concentrations in apprehended drivers were evaluated in relation to the typical therapeutic range of pregabalin as well as the age and gender of the driver. The concomitant use of other drugs of abuse in pregabalin positive cases was also assessed.

2. Material and methods

In 2012, the total number of requests for analysis of drug levels in DUID cases in Finland was 3863. All samples from drivers suspected of DUID underwent extensive screening for various psychoactive drugs. However, pregabalin analysis was only performed in cases where there was reason believe that the suspect had taken pregabalin, e.g. the suspect admitting taking the drug, or, pregabalin tablets were found on the suspect. The total number of cases where pregabalin was analysed was 459 which accounts for about 12% of the cases of suspected driving under the influence of drugs in that year. Serum samples were collected shortly after the incident and analysed for pregabalin by LC–MS/MS.

2.1. Chemicals and reagents

The reference standard for the quantitative determination of pregabalin was obtained from Pfizer (Berlin, Germany) and the internal standard 2-amino-3-cyclohexyl-1-propanolwas obtained from Fluka (Munich, Germany). HPLC grade acetonitrile was purchased from Baker (Griesheim, Germany) and ammonia 32% p.a. from Sigma (Munich, Germany).

2.2. Sample preparation and LC–MS/MS conditions

For the analysis 100 μL serum, calibrator or control were used. After protein precipitation by adding 500 μL of acetonitrile containing 10 mg/L 2-amino-3-cyclohexyl-1-propanol, the samples were centrifuged 10 min at 14000 rpm (10 °C). The QC standards were prepared in-house at concentrations 4 and 15 mg/L. The samples were analysed by an LC–MS/MS system consisting of a Waters Alliance 2795 HPLC device and a Waters Quattro Micro mass spectrometer (Waters, Eschborn, Germany). The separation column was Synergie max-rp 80A 50 × 3 mm from Phenomenex (Aschaffenburg, Germany). The mobile phase was ammonium acetate in acetonitrile (30/70 v/v). The injection volume was 20 μL, flow rate 0.3 mL/min and the column temperature 45 °C. The MRM transitions m/z 160 → 142 for pregabalin and m/z 158 → 123 for the internal standard were monitored in the analysis. LC–MS/MS chromatograms of a blank blood sample and samples spiked with pregabalin at two concentrations, all together with the internal standard, are presented in Fig. 2.

3. Method validation

The limit of detection (LOD) was found to be 0.22 mg/L based on the following criteria: CV ≤ 20%, accuracy 80–120% and $S/N \geq 3$, estimated from replicates of control samples (n = 10). Similarly, the limit of quantification (LOQ) for pregabalin was 0.68 mg/L, based on $S/N \geq 10$. These calculations were performed according to the German standard specification DIN 32645 [19,20].

Linearity was tested by the analysis of standards prepared in serum at concentrations between 0.68 mg/L and 20 mg/L. The mean value for each level was within 90–110% when a linear fit was used and thus the method was linear from 0.68 to 20 mg/L. For sample concentrations exceeding the calibration range a dilution with drug free serum was performed.

Dilution integrity was tested by determining analyte concentrations in 2- and 4-fold diluted samples prepared from serum samples spiked with pregabalin at low (3.92 mg/L) and high (15.43 mg/L) concentrations. The concentrations of the diluted samples were within 90–110% of that of the original sample.

For the determination of between-day precision, the quality controls (4 mg/L and 15 mg/L) were analysed on 10 days. The within-day precision of the method was assessed by calculating the coefficient of variation (CV) for 10 replicate analyses of the QC standards. Accuracy, expressed as percentage bias, was calculated as the percent difference between the amount of pregabalin added and found. The standard deviation of within-day repeatability was 4.5% at low level (4 mg/L) and 6.1% at high level (15 mg/L). The between-day precision was 5.5% over all. Repeatability of the assay was tested at 6 concentrations and the CV was within ±5% in all tested samples. The standard deviation (CV) of within- and between-day repeatability was between 6.1% and 10.3%. Accuracy ranged between 6.8% at 4 mg/L and 1.9% at 15 mg/L.

The matrix effect, measured in 6 different samples at concentrations 4 mg/L and 20 mg/L, showed an average enhancement of 20.4% (CV 8.7% and 8.1%, respectively). Recovery was 90.3%. Selectivity was determined by analysing pregabalin negative samples from routine.

Process stability was studied under the conditions of the LC–MS/MS analysis by analysing serum samples spiked with pregabalin over a period of 72 h. The tested concentrations were the same as in the dilution integrity test. The test solutions were stored in autosampler vials and kept cool at 10 °C between the runs. Pregabalin was stable for the studied time period (concentrations