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Tramadol detection in sports

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Abstract

Tramadol is a centrally acting synthetic opioid analgesic used similarly to codeine, to treat moderate to moderately severe pain. The drug has a wide range of applications, including treatment for Rheumatoid arthritis, restless legs syndrome and fibromyalgia. Tramadol possesses weak agonist actions at the μ-opioid receptor, releases serotonin, and inhibits the reuptake of norepinephrine. Opioids are chemical compounds which act upon one or more of the human opiate receptors. The opioid agonistic effect of tramadol and its major metabolite(s) are almost exclusively mediated by the substance's action at the μ-opioid receptor. This characteristic distinguishes tramadol from many other substances (including morphine) of the opioid drug class, which generally do not possess tramadol's degree of subtype selectivity. Tramadol has been included in 2012 in the WADA monitoring program and its potential abuse is suspected in sports. In order to investigate the urinary excretion of the best marker of tramadol administration, controlled administrations have been performed at single therapeutic dosages (100 mg of Tramadol Dorom®, p.o.) in healthy volunteers (n=3). Simultaneously the detection of tramadol intake has been reviewed in the 2011 samples analyzed in the WADA accredited Laboratory in Rome. On the view of monitoring tramadol and trying to establish a criteria for its potential abuse detection based on the detected concentration, it must be considered that a major part of its metabolisms is through a polymorphic enzyme. Further studies to assess this issue are in progress.

Introduction

Tramadol is rapidly and almost completely absorbed after oral or parenteral administration and is extensively metabolised via the hepatic cytochrome P450 isozyme CYP2B6, CYP2D6 (polymorphic) and CYP3A4, being O- and N-demethylated to five different metabolites and then conjugation with glucuronic acid and sulphate (Figure 1) [1,2].
Of these, O-desmethyltramadol is the most significant since it has 200 times the μ-affinity of (+)-tramadol, and furthermore has an elimination half-life of nine hours, compared with six hours for tramadol itself. About 90% of an oral dose is excreted in the urine in 3 days, about 40% of the dose as unchanged drug and the rest as metabolites.

Experimental

Tramadol was obtained from Cerilliant (LGC Standards, Milano, Italy) and diphenylamine (ISTD) was purchased from Sigma-Aldrich (Milano, Italy). All chemicals used were from Carlo Erba (Milano, Italy).

Excretion studies were performed on three healthy volunteers receiving a single oral dose of 100 mg of tramadol hydrochloride (Tramadolo Hexal ®, Hexal SpA, Monza e Brianza, Italy). The study was realized according to the Italian requirement for observational studies and informed consent was obtained from the volunteers. Spot urine samples were collected before administration and for 72 h after tramadol administration. The samples were stored frozen until analysis.

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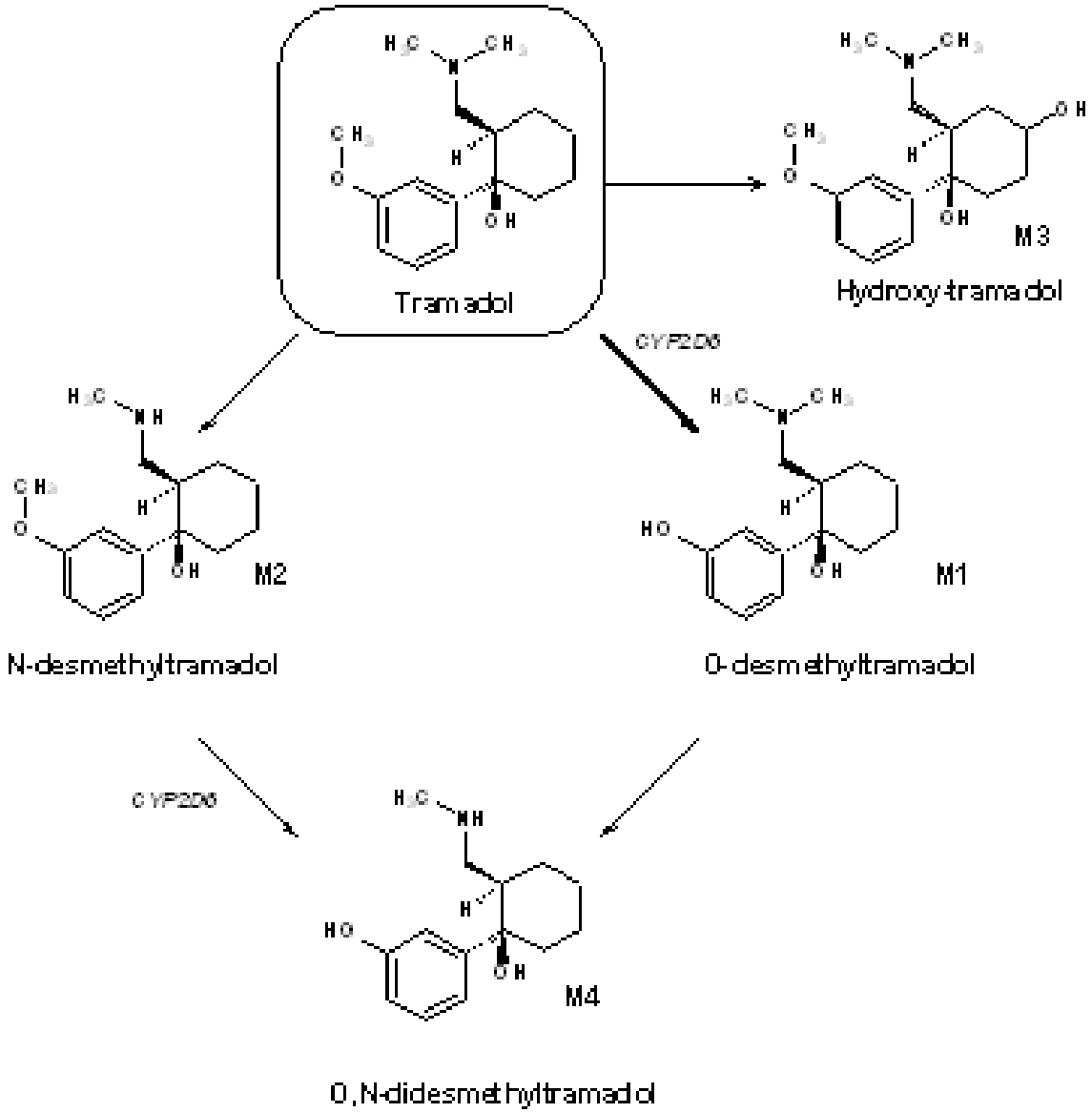

Figure 1: Tramadol metabolism scheme.

Sample preparation

Two mL of urine were alkalinized with 200 µL of NaOH 2M and NaCl (1g) added for salting out effect. The samples were extracted with 2 mL of tert-buthyl-methyl-ether. The organic extracts were taken to dryness under a reduced nitrogen flow at room temperature and reconstituted with 50 µL of extraction solvent before GC/MS analysis. Diphenylamine was added as internal standard (ISTD). The method is the current one used in the WADA accredited laboratory in Rome for the detection of stimulants and narcotics excreted unconjugated into urine.

Instrumental Analysis

The GC/MS analyses were performed on a Agilent GC 7890 interfaced with an Agilent 5975 MS (Agilent Technologies Italia S.p.A., Cernusco sul Naviglio, MI, Italy) operating in EI. The column was an HP5 (17m x 0.2 mm x 0.33 µm).
Oven program: 85°C (1 min), 15°C/min to 270°C, 50°C/min to 310°C (3.5 min).
Injection: 1 mL, pulsed splitless; Injector T: 270°C;
MS Acquisition: Full scan acquisition, mass range: m/z 50-335

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Results and Discussion

One therapeutic dose of tramadol (100 mg) was administrated to 3 healthy volunteers and the urine was analysed using the routine procedure for stimulants and narcotics to know the urinary excretion of tramadol. From these studies it appears that tramadol is excreted for about the 50% unchanged and that the major metabolite is O-desmethyltramadol (M1- 40%). The other metabolites, M2, M3 and M4, represent about 10% of the total (Figure 2). The analysis of the conjugated metabolites was not considered in this study to establish the simplest possible detection of the potential tramadol abuse. Tramadol metabolites identity was assigned based on already published reference spectra [2] and the MS fragmentation pattern. Tramadol metabolites were not quantified due to the lack of reference material and relative areas, based on the areas of base peak and internal standard, were plotted in order to compare the relative detection.

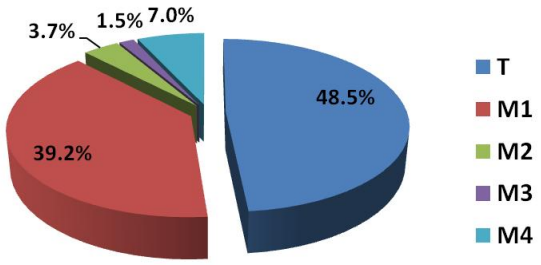

Figure 2: Percentage of detection of free tramadol and its metabolites (n=3).

The urinary peak of unconjugated tramadol appears at 10-12 h after intake and after 24-30 h tramadol is completely excreted (Figure 3). The metabolites excretion follows almost the same trend of excretion of tramadol (Figure 3), with an elimination half-life of about nine hours and the total excretion after ca. 30 h. From the analysis of the unconjugated metabolites, it appeared that the monitoring of unconjugated tramadol itself is the more convenient for the detection of tramadol abuse.

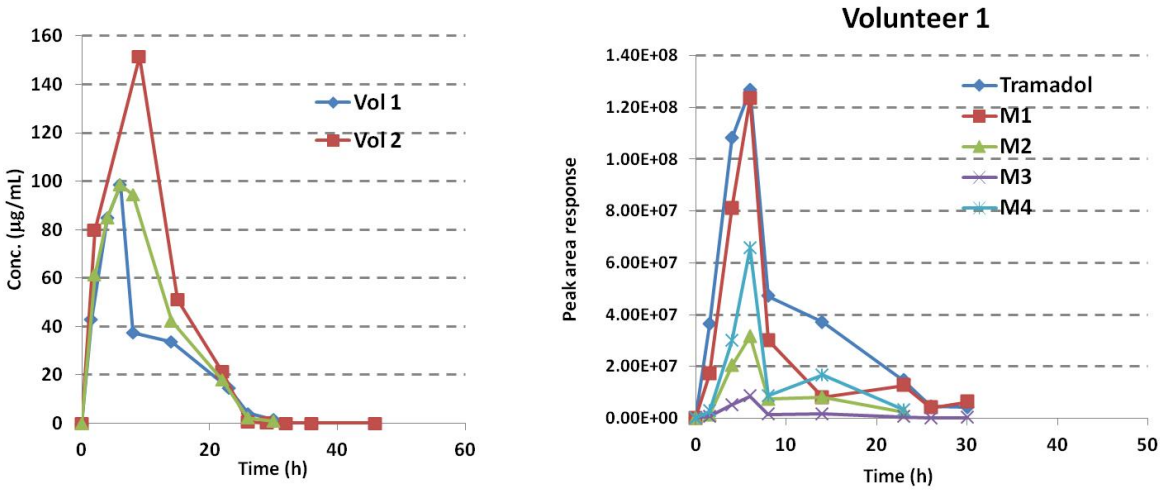

Figure 3: Time course of the urinary concentrations of free tramadol (n=3)(left) and time course of the urinary concentrations of free tramadol and its metabolites in volunteer 1 (right) after the oral administration of 100 mg of tramadol.

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Additionally, the detection of tramadol intake has been reviewed in the 2011 samples analyzed in the WADA accredited Laboratory in Rome. Data on the estimated concentrations and the spontaneous declaration of tramadol by the athletes has been recorded. From all the samples analysed (n=9593), 2.1% showed the presence of tramadol. Most of the cases detected were concentrated in cycling samples (92.4% of the cases) representing 13.4% of the all the cycling samples analysed.
From the comparison of the estimated concentrations it appears that the concentrations in athletes' real samples are compatible with the concentrations obtained after a single dose administration (Figure 4).

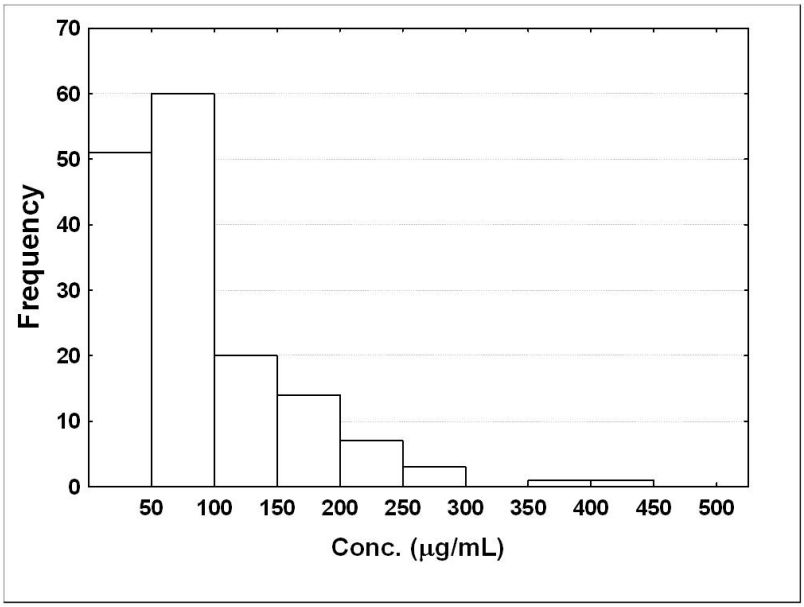

Figure 4: Frequency Distribution(%) of free tramadol concentration determined in 2011 routine urine samples (n=198).

Conclusions

From the excretion study it appears that, following oral administration, tramadol and its metabolites can be detected in the urine in free form and the unconjugated tramadol is a good marker of tramadol intake.
From the comparison of the estimated concentrations it appears that the concentrations of tramadol in routine urine samples are compatible with the concentration levels expected after a 100 mg single dose administration. It must be also noted that the consumption of this compound is concentrated in very specific sport disciplines. Since tramadol metabolism is through a polymorphic enzyme (CYP2D6), if a cut-off is established to detect a non therapeutic use, this should be considered on the evaluation of the concentrations obtained in a single athlete.

References

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