Toxicology and Applied Pharmacology 310 (2016) 108–119

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Mechanisms of tramadol-related neurotoxicity in the rat: Does diazepam/tramadol combination play a worsening role in overdose?☆

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ARTICLE INFO

Article history:
Received 19 July 2016
Revised 30 August 2016
Accepted 14 September 2016
Available online xxxx

Keywords:
Diazepam
Poisoning
Respiratory depression
Seizure
Serotonin
Tramadol

ABSTRACT

Poisoning with opioid analgesics including tramadol represents a challenge. Tramadol may induce respiratory depression, seizures and serotonin syndrome, possibly worsened when in combination to benzodiazepines. Our objectives were to investigate tramadol-related neurotoxicity, consequences of diazepam/tramadol combination, and mechanisms of drug-drug interactions in rats. Median lethal-doses were determined using Dixon–Bruce's up-and-down method. Sedation, seizures, electroencephalography and plethysmography parameters were studied. Concentrations of tramadol and its metabolites were measured using liquid-chromatography-high-resolution-mass-spectrometry. Plasma, platelet and brain monoamines were measured using liquid-chromatography coupled to fluorimetry. Median lethal-doses of tramadol and diazepam/tramadol combination did not significantly differ, although time-to-death was longer with combination ($P = 0.04$). Tramadol induced dose-dependent sedation ($P < 0.05$), early-onset seizures ($P < 0.001$) and increase in inspiratory ($P < 0.01$) and expiratory times ($P < 0.05$). The diazepam/tramadol combination abolished seizures but significantly enhanced sedation ($P < 0.01$) and respiratory depression ($P < 0.05$) by reducing tidal volume ($P < 0.05$) in addition to tramadol-related increase in respiratory times, suggesting a pharmacodynamic mechanism of interaction. Plasma M1 and M5 metabolites were mildly increased, contributing additionally to tramadol-related respiratory depression. Tramadol-induced early-onset increase in brain concentrations of serotonin and norepinephrine was not significantly altered by the diazepam/tramadol combination. Interestingly neither pretreatment with cyproheptadine (a serotonin-receptor antagonist) nor a benserazide/5-hydroxytryptophane combination (enhancing brain serotonin) reduced tramadol-induced seizures. Our study shows that diazepam/tramadol combination does not worsen tramadol-induced fatality risk but alters its toxicity pattern with enhanced respiratory depression but abolished seizures. Drug-drug interaction is mainly pharmacodynamic but increased plasma M1 and M5 metabolites may also contribute to enhancing respiratory depression. Tramadol-induced seizures are independent of brain serotonin.

Abbreviations: AUC, area under the curve; BZD, benzodiazepine; Cl, total clearance; C_{max}, peak concentration; CNS, central nervous system; DOPAC, 3,4-dihydroxyphenylacetic acid; DZP, diazepam; GABA, γ-aminobutyric acid; EEG, electroencephalogram;; *f*, respiratory frequency; 5-HIAA, 5-hydroxyindoleacetic acid; HPLC, high-performance liquid chromatography; HVA, homovanillic acid; IP, intraperitoneal; LC-HRMS, liquid chromatography-high resolution mass spectrometry; LD_{50}, 50% lethal dose;; MAO, monoamine oxidase; MLD, median lethal dose; MOR, mu-opioid receptor; MRT, mean residence time; PRP, platelet rich plasma; SC, subcutaneous; $T_{1/2\beta}$, terminal elimination half-life; T_E, expiratory time (T_E); T_I, inspiratory time; T_{max}, time to achieve the C_{max}; $V_{D/F}$, volume of distribution; V_M, minute volume; V_T, tidal volume.

☆ Conflict of interest statement: There are no conflicts of interest associated with this study.

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http://dx.doi.org/10.1016/j.taap.2016.09.013
0041-008X/

1. Introduction

To date, prescription opioids represent the leading cause of toxic death in the US, mainly attributed to deleterious behaviors of WHO step-2 analgesics (Dart et al., 2015). After dextropropoxyphene withdrawal from the market in the US (Nov 2010) and Europe (Sept 2011), tramadol use has dramatically expanded, raising the risk of increased poisonings and deaths attributed to this drug (Hawton et al., 2012).

In contrast to other opioids, tramadol-related anti-nociceptive activity is not only mediated by mu-opioid receptors (MOR) but also results from serotonin and norepinephrine reuptake inhibition, provided by both the parent drug enantiomers and *O*-demethyltramadol (M1), a metabolite with stronger MOR agonist activity (Grond and Sablotzki, 2004). Tramadol is responsible for life-threatening poisonings resulting in consciousness impairment (~30%), seizures (~15%), agitation (~10%) and respiratory depression (~5%) (Spiller et al., 1997). Like other opioids, central apnea has been attributed to the ingestion of elevated doses of tramadol (Hassanian-Moghaddam et al., 2013). By contrast, onset of seizures represents a major concern, tramadol accounting for about 8% of drug-induced seizures reported to poison control centers (Thundiyil et al., 2007). Tramadol poisoning typically result in isolated, brief, self-limiting generalized tonic-clonic seizures, most often occurring within 4–6 h postingestion (Jovanović-Cupić et al., 2006) but associated with common electroencephalogram (EEG) abnormalities (Shadnia et al., 2012). Serotonin syndrome characterized by neuromuscular hyperactivity, autonomic nervous system excitability and altered mental status may occur although its onset seems rare (Ryan and Isbister, 2015) and its impact underestimated (Tashakori and Afshari, 2010).

Tramadol poisoning often results from multidrug ingestion, generally involving benzodiazepines (BZD) (Spiller et al., 1997). Accordingly, tramadol-related fatalities have more often been attributed to a history of multidrug abuse with unintentional poisoning than suicide attempt, suggesting a major role of drug-drug interactions (Tjäderborn et al., 2007). An overrepresentation of tramadol among the serotonergic drugs causing death was also observed (Pilgrim et al., 2010). Deleterious consequences from tramadol in combination with BZD were suggested (Clarot et al., 2003), although the exact mechanisms of such drug-drug interactions are unknown.

To date, only rare experimental studies have investigated tramadol-induced toxicity. The relative contribution of seizures, central nervous system (CNS) depression and serotonin toxicity in the onset of tramadol-induced threat to life is poorly understood. While seizures are regarded as the major symptoms characterizing the serotonin syndrome, the exact relationships between both disorders in tramadol poisoning are controversial (Fujimoto et al., 2015; Ryan and Isbister, 2015; Tashakori and Afshari, 2010). Similarly, the contribution to tramadol toxicity of the parent drug versus its main metabolites is unclear. Finally, the consequences of the BZD/tramadol combination, often suggested to be deleterious, remain to be established. Thus, we conducted an experimental study in the rat aiming 1)- to describe tramadol-induced neurotoxicity alone and in combination with diazepam (DZP), one of the most frequently identified BZD in tramadol-attributed poisonings and fatalities (Tjäderborn et al., 2010); 2)- to investigate the mechanisms involved in DZP/tramadol drug-drug interactions; and 3)- to better understand the relationships between tramadol-induced seizurogenic and serotonergic effects.

2. Methods

Experimental protocols were carried out within the ethical guidelines established by the NIH and approved by Paris-Descartes University ethics committee for animal experimentation.

2.1. Animals and chemicals

Male Sprague-Dawley rats (Janvier) weighing 250–350 g at the time of experimentation were used, housed for 7 days before experimentation in an environment maintained at 21 ± 0.5 °C with controlled humidity and a light–dark cycle. Food and tap water were provided ad libitum.

Tramadol hydrochloride (Grünenthal) was diluted in sterile water to obtain a solution of 44 mg/ml. DZP (Roche, France) was diluted in 4% Tween (Tween-20® diluted in 0.9% NaCl) to obtain a solution of 20 mg/ml. Cyproheptadine (Teofarma), benserazide and 5-hydroxytryptophane (Sigma) were diluted in saline to obtain solutions of 8, 30 and 30 mg/ml, respectively.

2.2. Determination of tramadol median lethal dose (MLD)

We used the up-and-down method of Dixon–Bruce (Bruce, 1985) to limit the number of required animals, as follows: the first rat received 150 mg/kg tramadol, the estimated 50% lethal dose (LD_{50}) by intraperitoneal (IP) route in the rat (Poisindex®, Micromedex Healthcare Series, 2013). If the rat survived, the next animal received a 1.3-fold more elevated dose and so on until the trend changed (i.e. an animal died). Then, the next animal received a 1.3-fold less elevated dose than the previous lethal dose. This method progressively bracketed tramadol MLD, permitting the use of fewer animals than the classic MLD designs. Three series were used. Animals were observed at T0, 5, 10, 20, 30 min, then at 1, 1.5, 2, 3, 4, 5, 6, 7, 8 and 24 h after drug injection. The total number of required animals depended on the accuracy of the initial MLD estimation. The MLD was then determined according to the final tramadol dose, the chosen fixed percentage in the dose change, and the pattern of animal outcome at 24 h after the drug injection.

2.3. Femoral artery catheterization

The day before the study, the femoral artery was catheterized using 30 cm silastic tubing with external and internal diameters of 0.94 and 0.51 mm, respectively (Dow Corning Co., Midland, MI). The arterial catheter was tunneled subcutaneously and fixed at the back of the neck. Heparinized saline was injected into the catheter to avoid thrombosis and catheter obstruction. Rats were then returned to their individual cages for a minimum recovery period of 24 h, to allow complete anesthesia washout. On the day of experimentation, rats were placed in horizontal Plexiglas cylinders (6.5 cm-internal diameter, up to 20 cm-adjustable length) (Harvard Apparatus, Inc., Holliston, MA, USA), modified by the addition of several holes at the cephalic end to avoid CO_2 rebreathing (Chevillard et al., 2010). Before the drug administration, the catheter was exteriorized, purged, and its permeability checked.

2.4. Clinical findings

Temperature, sedation, and seizures were monitored by two researchers blinded to the group allocation. Temperature was measured using IP implanted temperature transmitters for the purpose of plethysmography study. Sedation level based on a 4-stage scale from 0 (awake) to 3 (coma) was assessed (Pirnay et al., 2008). At stage 0, rats were completely awake and their gait and righting reflexes were intact. At stage 1, rats had reduced activity, showed light impairment of gait and an intact righting reflex with diminished muscle tonus. At stage 2, rats were asleep or static and showed a reduced righting reflex. At stage 3, rats were comatose and did not have any righting reflex. Seizure severity was graded according to the modified Racine Score (Racine, 1972): At stage 1, rats were immobile, with eyes closed, twitching of vibrissae and facial clonus. At stage 2, rats had head nodding associated with more severe facial clonus. At stage 3, rats had clonus of one forelimb. At stage 4, rats had rearing, often accompanied by bilateral forelimb