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Effective analgesic doses of tramadol or tapentadol induce brain, lung and heart toxicity in Wistar rats

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ABSTRACT

Tramadol and tapentadol are extensively prescribed for the treatment of moderate to severe pain. Although these drugs are very effective in pain treatment, the number of intoxications and deaths due to both opioids is increasing, and the underlying toxic mechanisms are not fully understood. The present work aimed to study the potential biochemical and histopathological alterations induced by acute effective (analgesic) doses of tramadol and tapentadol, in Wistar rats. Forty-two male Wistar rats were divided into different groups: a control, administered with normal saline solution, and tramadol- or tapentadol-treated groups (10, 25 or 50 mg/kg – typical effective analgesic dose, intermediate and maximum recommended doses, respectively). 24 h after intraperitoneal administration, biochemical and oxidative stress analyses were performed in blood, and specimens from brain, lung and heart were taken for histopathological and oxidative stress studies. Both drugs caused an increase in the AST/ALT ratio, in LDH, CK and CK-MB activities in serum samples, and an increase in lactate levels in serum and brain samples. Oxidative damage, namely protein oxidation, was found in heart and lung tissues. In histological analyses, tramadol and tapentadol were found to cause alterations in cell morphology, inflammatory cell infiltrates and cell death in all tissues under study, although tapentadol caused more damage than tramadol.

Our results confirmed the risks of tramadol exposure, and demonstrated the higher risk of tapentadol, especially at high doses.

1. Introduction

Pain is a symptom associated with several pathologies, and opioids are currently used for the treatment of its moderate to severe forms. However, besides analgesia, the activation of μ-opioid receptors (MOR) also causes side effects such as central nervous system (CNS) depression, nausea, dependence and addiction (DePriest et al., 2015; Harrison et al., 1998; Kosten and George 2002). Hence, the use of non-classical opioids, which combine MOR agonist activity with monoamine reuptake blocking, has been seen to improve analgesia and decrease the side effects (Pergolizzi et al., 2012; Power 2011; Singh et al., 2013; Tzschentke et al., 2014; Vadivelu et al., 2010). Tramadol and tapentadol are two synthetic opioid analgesics, with a dual mechanism of action − opioid agonist action and monoamine reuptake inhibition (Barbosa et al., 2016; Mercier et al., 2014).

Tramadol (1*RS*, 2*RS*)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)-cyclo-hexanol is a racemic mixture of two enantiomers, (−)-tramadol and (+)-tramadol, that have two distinct but complementary mechanisms of action (Grond and Sablotzki 2004; Raffa et al., 2012). Tramadol is responsible for moderate MOR agonist activity, and noradrenaline (NA) and serotonin (5-HT) reuptake inhibition, and its analgesic activity is predominantly provided by the *O*-desmethyltramadol (M1) active metabolite (Duthie 1998; Gillen et al., 2000; Grond and Sablotzki 2004; Lai et al., 1996; Leppert 2011). Tapentadol (3-[(1*R*,2*R*)-

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3-(dimethylamino)-1-ethyl-2-methylpropyl]phenol) is a single and active molecule, without active metabolites (Bourland et al., 2010; Hartrick and Rozek 2011; Raffa et al., 2012). Tapentadol effect on the inhibition of 5-HT reuptake is less pronounced than that of tramadol, which reduces the risk of serotonin syndrome (Giorgi et al., 2012; Hartrick and Rozek 2011; Meske et al., 2014; Raffa et al., 2012; Steigerwald et al., 2013; Tzschentke et al., 2014).

Although tramadol and tapentadol have low incidence and intensity of side effects, addiction, respiratory depression and fatal cases have been reported (Clarkson et al., 2004; Costa et al., 2013; Dinis-Oliveira et al., 2012; Giorgi et al., 2012; Larson et al., 2012; Pinho et al., 2013; Ryan and Isbister 2015; Shadnia et al., 2008). Therefore, it is important to assess the potential toxicity of these compounds. Previous reports showed that acute gavage administration of tramadol LD_{50} induced rat brain alterations, including brain congestion, edema and inflammatory cellular infiltrates (Samaka et al., 2012). Chronic use of tramadol in increasing doses was also associated with neuronal degeneration in the rat brain, which has a possible contribution to cerebral dysfunction (Atici et al., 2005, 2004; Awadalla and Salah-Eldin, 2016). Repeated tramadol administration has also been shown to cause lung damage (Awadalla and Salah-Eldin, 2016). Moreover, *in vitro* acute exposure assays in a human neuronal model showed that both compounds, tramadol and tapentadol, cause toxicity (Faria et al., 2016). However, concerning tapentadol there are no studies evaluating its toxicity in *in vivo* models. Additionally, myocardial damage may be associated with the inhibition of NA reuptake (Vadivelu et al., 2011), and therefore it is important to evaluate the potential cardiovascular damage after tramadol and tapentadol exposure. Hence, it is relevant to assess the toxic effects of different doses, including overdoses, particularly concerning neurotoxicity and central nervous system dysfunction, and lung and heart damage, upon tramadol and tapentadol administration, in order to evaluate their safety. Therefore, the present work aimed to study biochemical changes, oxidative damage and histopathological toxicity in brain, lung and heart of rats exposed to a single dose, corresponding to a typical effective analgesic dose, an intermediate dose and the maximum recommended daily dose, of tramadol or tapentadol.

2. Materials and methods

2.1. Animals

In this study, 42 male Wistar rats were used. Animals (aged 8 weeks) were obtained from the IBMC – i3S Animal House (Oporto, Portugal), weighing between 250 and 275 g. Animals were kept in standard laboratory conditions (12/12 h light/darkness, 22 ± 2 °C room temperature, 50–60% humidity) for at least 1 week (quarantine) before starting the assays. Animals were allowed access to tap water and rat chow *ad libitum* during the quarantine period. Animal experimentation was performed accordingly to the Portuguese Agency for Animal Welfare (general board of Veterinary Medicine), in compliance with the Institutional Guidelines and the European Convention. All experimentation was conducted in conformity with ethical and human principles of research, complying with the current Portuguese laws and in line with the recommendations of the National Council of Ethics for the Life Sciences (CNECV).

2.2. Tramadol and tapentadol exposure

Animals were divided into 7 groups of 6 animals each. The different groups were exposed to different tramadol hydrochloride (Sigma-Aldrich) or tapentadol hydrochloride (Deltaclon) doses, by intraperitoneal (ip) administration. Group I was used as control, receiving normal saline solution (0.9% NaCl); groups II, III and IV were injected with 10, 25 and 50 mg/kg tramadol, respectively; groups V, VI and VII were injected with 10, 25 and 50 mg/kg tapentadol, respectively. 10, 25 or 50 mg/kg correspond to a typical analgesic dose, an intermediate dose and the maximum recommended daily dose, respectively, as will be discussed in the "Results" section. Tramadol and tapentadol doses were delivered in a volume of 1 mL of normal saline. After ip administrations, animals were individually housed in metabolic cages, for 24 h, and during the exposure period they were provided with water *ad libitum*, but not food, and monitored.

2.3. Surgical procedures and sample collection

Twenty-four hours after administrations, rats were sacrificed through anesthesia with sodium thiopental (B. Braun Portugal, 60 mg/Kg, ip). Then, blood samples were drawn by cardiac puncture, using a heparinized needle. Blood was centrifuged (3000*g*, 4 °C, 10 min) and serum was separated and stored (−80 °C) for further biochemical analysis (aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), creatine kinase isoform MB (CK-MB), glucose, lactate and lactate dehydrogenase (LDH)). Brain cortex, lungs and heart were removed, dried, weighed and processed.

2.4. Tissue processing for biochemical analysis

Brain cortex, lung and heart samples were homogenized using an Ultra-Turrax® homogenizer with 1:4 (m/v) of ice-cold 50 mM phosphate buffer (KH_2PO_4 + $Na_2HPO_4{\cdot}H_2O$), pH 7.4. Homogenates were centrifuged at 4000*g*, 4 °C, for 10 min. Supernatants were aliquoted and stored at −80 °C for protein, catalase (CAT) and superoxide dismutase (SOD) quantification and biochemical analyses (CK, lactate, LDH and proteins). 10% perchloric acid was added in a proportion of 1:1 to one aliquot of each homogenate. Then, samples were centrifuged at 13,000*g*, 4 °C, for 10 min. The supernatants were stored at −80 °C for the quantification of lipid peroxidation (LPO), and the pellets were used for quantification of carbonyl groups. Other aliquots of brain cortex, lungs and heart were homogenized, using an Ultra-Turrax® homogenizer, with 1:3 (m/v) of ice-cold isotonic buffer (300 mM sucrose, 10 mM HEPES, 2 mM EDTA) pH 7.9, and incubated for 15 min on ice, for further quantification of caspase 3 activity. After homogenization, the sample was vortexed and then centrifuged at 850*g*, 4 °C, for 10 min. The resulting supernatant was collected, and the pellets were resuspended in 500 µL ice-cold isotonic buffer, and again incubated for 15 min on ice and centrifuged at 14000*g*, 4 °C, for 10 min. The supernatant was collected and combined with the first supernatant. The supernatants were stored at −80 °C for protein and caspase 3 quantifications.

2.4.1. Biochemical analysis

2.4.1.1. Biochemical parameters. Biochemical parameters (AST, ALT, CK, CK-MB, lactate, LDH, glucose and proteins) were quantified in a Prestige 24i automated analyser (Tokyo Boeki) as previously described (Costa et al., 2015), using 2 appropriate calibrators, which were diluted to plot a standard curve with 5 points. A quality control was also used. Results were normalized against the control group, and also against protein content in brain samples.

2.4.1.2. Measurement of toxicity biomarkers. LPO was evaluated using the thiobarbituric acid-reactive substances (TBARS) methodology (Buege and Aust, 1978) and the results were expressed as nanomoles of malondialdehyde (MDA) equivalents per gram of protein. Protein carbonyl groups (ketones and aldehydes) were measured according to Levine et al. (Levine et al., 1994), with the results being expressed as nanomoles of 2,4-dinitrophenylhydrazine (DNPH) incorporated per gram of protein. CAT and SOD activity was determined according to the method of Aebi (Aebi, 1984) and of McCord and Fridovich (McCord and Fridovich, 1969), respectively. The results were expressed in enzyme units per gram of protein and were normalized against the control group. Caspase 3 activity was determined as described by Faria et al. (Faria et al., 2016), using caspase 3 peptide substrate Ac-DEVD-