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RESEARCH PAPER

# Preliminary pharmacokinetics of tramadol hydrochloride after administration via different routes in male and female B6 mice

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## Abstract

**Objective** 1) Determine the pharmacokinetics of tramadol hydrochloride and its active metabolite, *O*-desmethyltramadol (M1), after administration through different routes in female and male C57Bl/6 mice; 2) evaluate the stability of tramadol solutions; 3) identify a suitable dose regimen for prospective clinical analgesia in B6 mice.

**Study design** Prospective, randomized, blinded, parallel design.

**Animals** Eighteen male and 18 female C57Bl/6 mice (20–30 g).

**Methods** Mice were administered 25 mg $kg^{-1}$ tramadol as a bolus [intravenously (IV), intraperitoneally (IP), subcutaneously (SQ), orally per gavage ($OS_{gavage}$)] over 25 hours [oral in drinking water ($OS_{water}$) or Syrspend SF ($OS_{Syrsp}$)]. Venous blood was sampled at six predetermined time points over 4 to 31 hours, depending on administration route, to determine tramadol and M1 plasma concentrations (liquid chromatography and tandem mass spectrometry detection). Pharmacokinetic parameters were described using a non-compartmental model. The stability of tramadol in water (acidified and untreated) and Syrspend SF (0.20 mg $mL^{-1}$) at ambient conditions for 1 week was evaluated.

**Results** After all administration routes, $C_{max}$ was >100 ng $mL^{-1}$ for tramadol and >40 ng $mL^{-1}$ for M1 (reported analgesic ranges in man) followed by short half-lives (2–6 hours). The mean tramadol plasma concentration after self-administration remained >100 ng $mL^{-1}$ throughout consumption time. M1 was found in the $OS_{Syrs}$ group only at 7 hours, whereas it was detectable in $OS_{water}$ throughout administration. Tramadol had low oral bioavailability (26%). Short-lasting side effects were observed only after IV administration. Water and Syrspend SF solutions were stable for 1 week.

**Conclusions and clinical relevance** 1) At the dose administered, high plasma concentrations of tramadol and M1 were obtained, with half-life depending on the administration route. 2) Plasma levels were stable over self-consumption time. 3) Solutions were stable 1 week at ambient conditions.

***Keywords*** analgesia, mice, pharmacokinetics, tramadol.

## Introduction

Mice are widely used as laboratory models for surgical procedures. The provision of appropriate analgesia for peri- and postoperative pain is an ethical and legal imperative (Carbone 2011) and essential for



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scientific integrity as untreated pain is expected to affect the outcome data. However, providing an effective analgesic treatment for the target species is challenging for involved scientists (i.e. veterinarians, researchers, animal welfare bodies) because of the biological peculiarities, the sparse published data of both the pharmacokinetics and efficacy of potentially relevant analgesics in the target species or strain, and finally the potential for interaction with the experimental readout.

Mice as a prey species tend to hide signs of pain, which hampers the recognition and quantification of pain, contributing to the underuse of postoperative analgesics. To date, the spectrum of analgesics available for laboratory mice relies mainly on few opioids (i.e. buprenorphine) and non-steroidal anti-inflammatory drugs (NSAIDs; carprofen, meloxicam). While offering potentially good analgesic options for mice (Tubbs et al. 2011; Oyama et al. 2012; Jirkof et al. 2015), they have limitations. NSAIDs are accompanied by anti-inflammatory and immune-modulatory effects (Iñiguez et al. 1999; Paccani et al. 2002); hence, are inappropriate for studies involving inflammation and the immune system. Additionally, their efficacy is questionable based on the latest evidence (Roughan et al. 2016).

The μ-agonist opioids, apart from interfering with the immune response to some extent (Page 2005; Franchi et al. 2007; Ricardo Buenaventura et al. 2008), present dose-dependent undesirable side effects, such as respiratory and gastrointestinal depression, tolerance, hyperalgesia or increased activity (Flecknell 1984; Hayes et al. 2000; Hau & Schapiro 2002; Ricardo Buenaventura et al. 2008; Grimm et al. 2015).

As a result of its relatively high benefit/risk ratio, favourable pharmacokinetic properties, low potential for drug interactions in humans and other animal species (Lewis & Han 1997), and non-controlled substance schedule, tramadol might be an interesting candidate to widen the analgesic portfolio in mice. Actual evidence of the analgesic efficacy of tramadol in mice is controversial: a recent study demonstrated that tramadol ameliorates cyclophosphamide-induced bladder-pain-related behaviours in mice (3–10 mg $kg^{-1}$, orally) (Oyama et al. 2012), whereas Wolfe et al. (2015) do not recommend it as a sole analgesic after abdominal laparotomy in mice. However these studies had no pharmacokinetic (PK) profiles supporting the dynamic data.

Pain treatment can be further improved by optimizing the methods of administration, long-lasting methods with the least stress possible (i.e. sustained/controlled release formulations or self-administration methods). Self-administration methods are an attractive option because they could ensure stable drug levels in the blood, which is necessary for adequate (in both intensity as duration) analgesic coverage while avoiding repetitive handling of the animals, thereby minimizing the stress. Therefore, in recent years different types of vehicles (water, pellets, Nutella, jelly) have been tested for voluntary oral drug delivery in laboratory animals with some success. There are already available data supporting this route of administration for analgesics (i.e. buprenorphine) in rats and mice (Abelson et al. 2012; Molina-Cimadevila et al. 2014); however, to the best of our knowledge, there is no published data regarding tramadol delivery in mice by such means.

Based on the rationales expressed and knowledge gaps in the literature, this study aims to: 1) determine the pharmacokinetics of tramadol and M1 after tramadol administration through different routes in female and male B6 mice; 2) evaluate the stability of tramadol in aqueous solution, to explore the feasibility of using drinking water for tramadol delivery; and 3) determine the most suitable route or combination of routes for this strain.

## Materials and methods

### Animals

The experimental protocol was approved by the local veterinary authorities (Kanton BS, TVB # 2768). Based on the planned PK profile design, 18 male and 18 female C57BL/6J mice [20–30 g body weight (BW)] were used, the rationale behind this number of mice was to use the minimum amount of mice necessary that allowed performing the analysis. Inclusion criteria were: strain, age and BW, healthy on clinical examination and based on review of health reports [according to Federation of European Laboratory Animal Science Associations (FELASA) health monitoring recommendations] (Mähler et al. 2014). Exclusion criteria were as follows: failure to adhere to pre-test requirements or overt sign of illness.

Mice were housed in groups of three animals in standard polycarbonate cages, with aspen wood bedding (J. Rettenmeier & Söhne GmbH, Germany) and nesting material. A rotational enrichment plan was in place, with hemp rope (Cordag AG,





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