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Research Paper

# Tramadol:Paracetamol in drinking water for treatment of post-surgical pain in laboratory mice

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ABSTRACT

In the search for stress-free analgesia administration for laboratory mice suffering pain, oral delivery of Tramadol:Paracetamol (T:P) shows great promise. Here, we monitored the analgesic efficacy and side effects of a T:P combination administered solely in the drinking water of female C57BL/6J mice after moderate-impact surgery (sham embryo transfer), using clinical and behavioral pain and recovery parameters. Animals underwent anesthesia and surgery with T:P treatment (OP + T:P), received no pain relief after surgery (OP), underwent anesthesia only with T:P treatment (An + T:P) or T:P treatment only (T:P). Indicators of pain and constraint were assessed at several time points during 24 h after surgery.

T:P-containing drinking water was consumed readily in amounts to assure sufficient drug levels. No obvious detrimental side effects of analgesia were observed. General condition of animals differed only slightly and non-significantly between treatment groups, with comparable post-procedural weight loss, water and food intake as well as home cage activity. Mean nest scores differed significantly between T:P and both surgery groups ($p = 0.002$, $p < 0.0001$) but revealed no significant difference between OP and OP + T:P groups. Nevertheless, pain scores showed significant differences between the treatment groups at 1, 3 and 6 h after surgery ($p = 0.001$, $p = 0.014$, $p = 0.003$). OP animals scored highest, while scores of OP + T:P animals were comparable or lower than scores of the AN + T:P group. Same was true for burrowing latency that was significantly increased in OP animals compared to An + T:P and OP + T:P ($p = 0.032$, $p = 0.019$), but comparable between An + T:P and OP + T:P. These results hint on a clear post-surgical pain effect after surgery that could be significantly reduced with T:P treatment towards a level of the control group receiving anesthesia and T:P only.

In conclusion, we assume that orally administered T:P offers pain relief with no obvious side effects after mild-to-moderate impact surgery in female C57BL/6J mice.

## 1. Introduction

In all animal experiments involving painful procedures or conditions, effective pain relief is a prerequisite for (1) good animal welfare, (2) reduction of animal numbers required and (3) high quality scientific results. Nevertheless, the assessment of pain, especially mild-to-moderate pain, can be challenging in laboratory mice. Therefore, scientific evidence for the efficacy of analgesia protocols in mice, even those most commonly used, is hard to find in the literature. Despite extensive knowledge of multiple pain types, sources and pathways, protocols for multimodal therapies such as those used commonly in humans or in large laboratory species are particularly scarce for mice. Additionally, unwanted side effects on scientific read-outs exclude the use of NSAIDs or certain opioids in some experimental settings in small rodents (GV-SOLAS, 2015; Jirkof et al., 2015).

Repeated injection of analgesics, which requires restraint and manipulation of the animal, can inflict additional stress (Meijer et al., 2005, 2006; Cinelli et al., 2007; Jirkof et al., 2015). Thus, recent publications have focused on continuous and/or self-administration analgesic protocols, such as sustained release formulations (Carbone et al., 2012; Healy et al., 2014; Jirkof et al., 2015), or the provision of analgesia via Nutella ® (Goldkuhl et al., 2008), gel delivery systems (Hovard et al., 2015) or in the regular diet (Molina-Cimadevila et al., 2014). The administration of analgesia in drinking water is an especially promising and simple way of providing continuous and stress-free pain treatment in mice (Hayes et al., 2000; Jessen et al., 2007).

A Tramadol:Paracetamol (T:P) formulation combines analgesics of different classes, and might represent a promising candidate for analgesic administration via drinking water in mice suffering mild to moderate pain. T:P is available commercially as tablets and is used

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frequently and effectively in human patients suffering a wide range of different pain states, including post-surgical pain, dental pain, and different types of chronic pain such as, for example, lower back pain (McClellan and Scott, 2003; Ruoff et al., 2003; Raffa, 2006; Emir et al., 2010; Alfano et al., 2011; Park et al., 2015).

Tramadol hydrochloride is a synthetic analgesic drug used in moderate-to-severe pain states. It exhibits opioid and non-opioid properties, and acts mainly on the central nervous system. Tramadol acts on μ-opioid and κ-opioid receptors with low affinity, exerting a weak agonist effect, and affects the monoamine system by blocking the norepinephrine and serotonin reuptake responsible for the inhibition of pain transmission in the spinal cord (Vazzana et al., 2015). Paracetamol (Acetaminophen) is an effective low-potency analgesic and antipyretic agent with a favorable safety and tolerability profile, acting at spinal and supra spinal sites (Raffa, 2006). Although the mechanisms underlying the effect of paracetamol remain uncertain, it is known to lack anti-inflammatory or coagulation effects (Tauben, 2015).

Administered in combination, these two drugs, with well-established complementary pharmacokinetics and mechanism of action, show a synergistic or additive effect in humans (Schug, 2006). This potential advantage over monotherapy results in maximized analgesic effect and minimized side effects as reduced amounts of each drug are needed for pain relief. The lack of gastro-intestinal, renal and cardiovascular effects, as well as there being no impact on platelets and no occurrence of immunosuppression (Schug, 2006) make T:P a welcome alternative to COX-2 selective or non-selective NSAIDs in the treatment of pain.

These synergistic or additive effects resulting in enhanced analgesia have also been described in studies using thermal and chemical pain stimuli in mice and rats (Hama and Sagen, 2010; Gong et al., 2011; Zhang et al., 2011; Miranda et al., 2012). However, despite its frequent use after surgery in humans, no scientific studies evaluating analgesic efficacy of oral T:P in a clinically relevant setting have been conducted in rodents to date.

We hypothesize that oral self-administration of a T:P combination provides measurable pain relief after surgery in female mice. Therefore, we analyzed the analgesic efficacy and side effects of a T:P combination after a standard moderate-impact surgery, using clinical and behavioral pain and recovery parameters.

## 2. Methods

### 2.1. Ethics statement

The animal housing and experimental protocols were approved by the Cantonal Veterinary Office, Zurich, Switzerland, under license no. ZH 181/2012 and ZH 059/2017, and were in accordance with Swiss Animal Protection Law. Housing and experimental procedures also conform to European Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals used for Scientific Purposes and to the Guide for the Care and Use of Laboratory Animals (Worlein et al., 2011).

### 2.2. Animals and standard housing conditions

The animals were 64 female C57BL/6J mice obtained from a commercial supplier (Charles River France) at the age of 6–8 weeks. We used solely female mice for better comparability with our own published and unpublished data. A health surveillance program according to FELASA guidelines throughout the experiments monitored the animals' health status. The mice were free of all viral, bacterial, and parasitic pathogens listed in FELASA recommendations (Mahler Convenor et al., 2014).

All animals were housed in groups of three to six animals for at least 3 weeks prior to testing in our animal room. Animals were kept in Eurotype III clear-transparent plastic cages (425 mm × 266 mm × 155 mm) with autoclaved dust-free sawdust bedding and autoclaved hay as nest building material (Winzeler, Affoltern, Switzerland). They were fed a pelleted and extruded mouse diet (Kliba No. 3436, Provimi Kliba, Kaiseraugst, Switzerland) ad libitum and had unrestricted access to sterilized drinking water. The light/dark cycle in the room consisted of 12/12 h with artificial light (approximately 40 lx in the cage). The temperature was 21 ± 1 °C, with a relative humidity of 55 ± 10%, and with 15 complete changes of filtered air per hour (HEPA H 14 filter). The animal room was insulated to prevent electronic and other noise. Disturbances, e.g., unrelated experimental procedures in the animal room, were not allowed.

### 2.3. Experimental design, experimental animal housing conditions, and data acquisition

#### 2.3.1. Analgesia protocol

The dosages for Tramadol and Paracetamol in drinking water were based on the recommendations of GV-SOLAS (GV-SOLAS, 2015) [1 mg Tramalhydrochlorid (Tramalhydrochlorid 100 mg/mL; Grünenthal Pharma AG, Glarus Süd, Switzerland) per milliliter drinking water], and on a widely used protocol (Flecknell, 2000) [5 mg Paracetamol (Dafalgan Kinder-Sirup, 30 mg/mL, Upsamedica, Baar, Switzerland) per milliliter drinking water]. Thus, 1 mL of drinking water offered to the animals contained 1 mg Tramadol and 5 mg Paracetamol.

#### 2.3.2. Experiments and data acquisition

Mice were housed individually in two specific settings for 3 days before and 2 days after the experimental intervention. Mice were housed in a standard cage containing a burrowing apparatus (a food pellet filled water bottle, see also (Jirkof et al., 2010)) to investigate burrowing performance and clinical condition. For automated activity tracking and determination of food and water intake, as well as for body mass measurements, mice were housed in a special observation cage (a standard Eurotype III clear-transparent plastic cage (425 mm × 266 mm × 155 mm) with raised walls (620 mm, clear-transparent plastic) instead of a cage grid); bedding and one nestlet (5 cm × 5 cm) consisting of cotton fibers (Indulab AG, Gams, Switzerland) was provided. Animals received T:P-treated water bottles overnight before the first experimental investigation and intervention, and on the following day.

For each experimental housing condition (burrowing set-up or observation cage), eight mice were allocated randomly (by simple randomization using specialized software) to one of four experimental groups: T:P in the drinking water only (T:P), anesthesia and T:P in the drinking water (An + T:P) or mice undergoing anesthesia and surgery with or without T:P in the drinking water (OP + T:P; OP).

*2.3.2.1. Surgical procedure.* Animals were transferred in transport cages (i.e. standard cages with a filter top) to a nearby operating theatre 1 h before lights on. The same experienced surgeon (MA) conducted all procedures. Mice were anaesthetized with sevoflurane (Sevorane, Abbott, Baar, Switzerland) as mono-anesthesia. The anesthetic gas was provided with a rodent inhalation anesthesia apparatus (Provet, Lyssach, Switzerland); oxygen was used as carrier gas. After induction of anesthesia in a Perspex induction chamber (7–8% sevoflurane, 600 mL/min gas flow), animals were transferred to a warmed (39 ± 1 °C) operating table, and anesthesia was maintained via a nose mask. Eye ointment was applied, the fur was clipped and the operating field disinfected with ethanol (70%) in all animals. Mice in surgery groups underwent a one-side sham embryo transfer. The incision in the abdominal muscle wall was closed with absorbable sutures (Vicryl, 6/0 polyglactin 910, Ethicon Ltd., Norderstedt, Germany), and the skin was closed using skin staples (Precise, 3M Health Care, St. Paul, MN, USA). Surgery was completed within 3–4 min in the surgery groups. Anesthesia lasted 6–8 min. While regaining consciousness after anesthesia, animals stayed for ~10 min on the warmed table before being transferred back to the animal room.