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Buprenorphine via drinking water and combined oral-injection protocols for pain relief in mice

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ABSTRACT

Buprenorphine is the opioid analgesic most commonly used in laboratory mice. However, to maintain therapeutically effective serum levels, repeated injections are required. To overcome negative aspects of restraint and injection, oral self-administration is a promising alternative but has been criticized to be unreliable. Here we analyze voluntary intake of buprenorphine via drinking water as well as drinking water/injection combinations for their reliability to achieve effective drug supply in C57BL/6J female mice.

Mice were assigned to one of five groups: a) naïve/no treatment (*N*); b) buprenorphine administration via drinking water for 24 h (*W*); c) buprenorphine administration via two subcutaneous injections during light, and via drinking water during dark phase (*IW2*); d) buprenorphine administration via three subcutaneous injections during light phase and drinking water for 24 h (*IW3*) or e) surgery plus buprenorphine administration via three subcutaneous injections during light phase and drinking water for 24 h (*S*). Drinking frequency, water and food intake, activity, body mass progression, blood serum concentrations of buprenorphine and behavioral pain indicators were determined.

Water intake was not decreased due to buprenorphine treatment or surgery. Administration of buprenorphine resulted in a significant increase of home cage activity in *IW3* animals and a decrease in body mass (n.s.). Food intake decreased significantly in *IW2*, *IW3* and *S*, compared to naïve mice (*IW2*: $p = 0.001$; *IW3*: $p = 0.0253$; *S*: $p \leq 0.0001$). All treatment groups showed mean serum concentrations higher than the targeted value (>1 ng/ml) throughout dark phase.

Nevertheless, sporadic drinking events and consequently highly variable individual serum concentrations during light phase suggest the use of a combination protocol (*IW3*: 24 h water administration + injections every 4 h during light phase), that proved to result in continuous therapeutic mean and individual serum concentrations and minimization of pain indicators after surgery (*S*).

1. Introduction

Ethical, legal and scientific considerations require the effective prevention and treatment of pain in laboratory animals (Institute of Laboratory Animal Resources (U.S.) Committee on Pain and Distress in Laboratory Animals, 1992).

Today, buprenorphine is one of the most widely used opioid analgesics in the treatment of pain in laboratory and companion animals (Roughan and Flecknell, 2002). It is fast acting and potent, with mixed agonist-antagonist activity at classical opioid receptors and has been shown to be effective in a variety of pain models (Christoph et al., 2005). However, to maintain therapeutically effective serum levels in mice, injections may be required more than four times in 24 h (Jirkof et al., 2015).

Repeated post-surgical injections of analgesic drugs require restraint and manipulation of the animal. Handling and restraint alone may impose stress even on healthy animals (Meijer et al., 2006; Cinelli et al., 2007), and are assumed to evoke additional pain, or to increase existing pain in animals with fresh surgical wounds (Jirkof et al., 2015). Both the lack of efficient post-surgical pain treatment and additional handling/restraint might induce a stress response, which will have effects on physiological and endocrine function and therefore might impair the recovery of the animals.

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This stress response may be a significant confounder of experimental data, leading to imprecise results and therefore to increased inter- and intra-animal variation (Moberg, 1999).

Attempts have been made to overcome these problems and to assure continuous and stress-free administration of buprenorphine analgesia. Several authors have described depot formulations of analgesia for rodents (Foley et al., 2011; Carbone et al., 2012; Healy et al., 2014; Jirkof et al., 2015). For example, Jirkof et al. (Jirkof et al., 2015) presented a sustained release formulation of buprenorphine that offers a long-lasting, assured blood concentration, resulting in an anti-nociceptive effect, and suggested relief of post-surgical pain for 24–48 h, without causing additional stress to the animals. However, while sustained-release formulations of buprenorphine have become commercially available on the US market (Animalgesics ® for Mice, Animalgesic Labs Inc, Millersville, MD, USA; Buprenorphine HCl CIII SR, Wildlife Pharmaceuticals Inc, Windsor, CO, USA), they are not available in Europe to date.

Oral self-administration of buprenorphine is another promising approach to administering analgesia without the negative effects of handling. Nevertheless, oral self-administration has been criticized as less effective than subcutaneous treatment in rats (Martin et al., 2001; Thompson et al., 2004; Thompson, et al., 2006) and compromised bioavailability due to first-pass metabolism, referring to reduced drug concentration due to the drug being metabolized before it reaches systemic circulation, is a known obstacle in this administration route (Brewster et al., 1981). Despite these concerns, several studies in mice and rats have shown that buprenorphine has sufficient analgesic efficacy when administered orally. Several routes of oral administration have been described, such as mixing buprenorphine with flavored gelatin (Liles et al., 1998), Nutella ® (Goldkuhl et al., 2010; Kalliokoski et al., 2011), gel delivery systems (Hovard et al., 2015) or with the regular diet of the mice (Molina-Cimadevila et al., 2014).

While these routes of administration have been shown to provide analgesia, they also have their limitations. For instance, food neophobia is a well-known obstacle in the oral administration of analgesics in mice. Habituation to new food items is necessary in order to ensure sufficient intake and resulting therapeutic drug levels (Liles et al., 1998; Goldkuhl et al., 2010; Kalliokoski et al., 2011; Hovard et al., 2015). Moreover, even after habituation, the latency to ingestion of the drug, as well as the total amount ingested by the animals, might be difficult to anticipate (Hovard et al., 2015). Providing buprenorphine mixed with the regular diet might overcome the problem of food neophobia, as stated by Molina-Cimadevila et al. (Molina-Cimadevila et al., 2014). Nonetheless, medicated food items need to be prepared prior to administration, which might be costly and time consuming depending on the chosen food medium (Liles et al., 1998; Goldkuhl et al., 2010; Kalliokoski et al., 2011; Hovard et al., 2015). Alternatively, providing analgesia mixed with drinking water is a promising route of administration (Hayes et al., 2000; Jessen et al., 2007) since tap water is readily available at every facility and mixtures can be prepared within minutes.

The present study aimed to explore whether administering buprenorphine in drinking water offers a reliable treatment option for pain management in mice or if a combination with buprenorphine injections may be necessary for reliable drug supply. In a first experiment three analgesic protocols were tested: administration via drinking water (*W*), a combination of two buprenorphine injections during the light phase and administration via drinking water in the dark phase (*IW2*) and a combination of three buprenorphine injections during the light phase and administration via drinking water for 24 h (*IW3*). Drinking behaviour, spontaneous water and food intake, blood serum concentrations reached by the drug over time, and behavioural modifications possibly evoked by the drug were assessed.

We hypothesize that laboratory mice drink the buprenorphine treated water regularly and in sufficient amounts, at least during the dark phase, to reach continuous therapeutic buprenorphine serum concentrations and also to minimize pain indicators after one-side sham embryo transfer. We therefore tested in a second experiment the most promising analgesic protocol (*IW3*) for its reliability in assuring pain relief in surgically treated mice, using clinical investigation and behaviour-based pain assessment.

2. Materials and methods

2.1. Ethics statement

The animal housing and experimental protocols were approved by the Cantonal Veterinary Office, Zurich, Switzerland, under license no. 181/2012, and were in accordance with Swiss Animal Protection Law and 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 110 female C57BL/6J mice obtained at the age of 4–5 weeks (Charles River, Sulzfeld, Germany). Their health status was monitored by a health surveillance programme according to FELASA guidelines. The mice were free of all viral, bacterial, and parasitic pathogens listed in FELASA recommendations, except for *Helicobacter* species (Mahler et al., 2015).

Mice were housed in groups of four to eight animals for three weeks prior to testing. All animals were maintained in Eurotyp III clear transparant plastic cages (Techniplast, Hohenpeissenberg, Germany) with a 12-h light/dark cycle (lights on at 8:00) with artificial light (approximately 40 lx in the cages), controlled temperature and relative humidity of $21 \pm 1\,^{\circ}C$ and $55 \pm 10\%$. They were fed a pelleted and extruded mouse diet (Kliba No. 3436, Provimi Kliba, Kaiseraugst, Switzerland) ad libitum and had unrestricted access to drinking water. Autoclaved dust-free sawdust bedding (80–90 g/cage; LTE E-001 Abedd, Indulab), autoclaved hay (8–12 g/cage; Winzeler, Affoltern am Albis, Switzerland) and one nestlet™ (5 cm × 5 cm), consisting of cotton fibres (Indulab AG, Gams, Switzerland), as nesting material and cardboard shelters (Ketchum Manufacturing, Brockville, Canada) were provided.

2.3. Treatment protocols

Naïve mice (N): Naïve mice received tap water for the experimental period. *Buprenorphine administration via drinking water (W):* Temgesic (Temgesic solution, 0.3 mg/ml, Reckitt Benckiser, Switzerland), a water-soluble buprenorphine medicinal product, was administered in the drinking water of the mice. Temgesic was diluted using tap water to a dose of 0.009 mg/ml drinking water. At the beginning of the light phase (8:00), mice were provided with a freshly prepared bottle of buprenorphine-treated water. The dose of buprenorphine was chosen to be approximately 10 times higher than the subcutaneous dose (Liles et al., 1998; Roughan and Flecknell 2002), assuming that a mouse would drink approximately 3 ml of the buprenorphine-treated water per day.

Buprenorphine administration via two injections during light phase and drinking water during dark phase (IW2): Mice were injected subcutaneously twice at a commonly used dose of 0.1 mg buprenorphine/kg body mass, at 4 h (12:00) and 10 h (18:00) after the beginning of the light phase. Shortly before the injection, Temgesic was diluted in sterile NaCL (0.9%) so that the injection volume was 2 μl/g body mass. Following the second injection, the animals were provided with buprenorphine-treated drinking water overnight,

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