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Serum concentrations of buprenorphine after oral and parenteral administration in male mice

Otto Kalliokoski [a], Kirsten R. Jacobsen [a], Jann Hau [a], Klas S.P. Abelson [a,b,*]

[a] Department of Experimental Medicine, University and University Hospital of Copenhagen, Copenhagen, Denmark
[b] Department of Neuroscience, Division of Comparative Medicine, Uppsala University, Uppsala, Sweden

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ABSTRACT

Buprenorphine is the most commonly used drug for peri-operative pain relief in laboratory rodents. The systemic concentrations of buprenorphine were measured in mice following administration intravenously (IV), subcutaneously (SC), orally by gavage and by voluntary ingestion, to determine the post-administration serum concentration of buprenorphine. Voluntarily ingested buprenorphine resulted in long-lasting high serum concentrations, as did oral gavage administration (24 h serum concentration: 110 ng h/mL for both routes of administration). In contrast, buprenorphine administered parenterally remained in the circulation for a substantially shorter time (24 h serum concentration for IV and SC were 40 ng h/mL and 30 ng h/mL, respectively). This marked difference was probably due to the higher dose used for oral administration, which is regarded necessary for sufficient analgesic effect, and to the slower absorption of the oral boli, as well as saturation of the hepatic buprenorphine metabolising pathways. Voluntary ingestion of buprenorphine was found to constitute a practical way to provide laboratory mice with efficient pain relief.

Introduction

Pain and stress in laboratory animals are a concern as they can influence experimental results and are animal welfare issues. It is therefore important to minimise pain and stress in animals subjected to surgical procedures. Buprenorphine is an opioid analgesic with agonistic and antagonistic effects on μ- and κ-opioid receptors, respectively (Cowan et al., 1977a,b; Leander, 1988), and it is frequently used for the treatment of post-operative pain in several animal species, including rodents (Dobromylskyj et al., 2000; Stokes et al., 2009). The most common route for administration is via intravenous (IV) or subcutaneous (SC) injection. However, this involves restraint and transient pain, which may be unnecessarily stressful to the animals. To minimise the stress response related to the administration procedure, it is desirable to develop procedures not dependent on restraint of the animal.

In rats, buprenorphine will induce analgesia in analgesiometric tests (Flecknell, 2001; Roughan and Flecknell, 2002), with a duration of approximately 6–12 h (Gades et al., 2000; Dobromylskyj et al., 2000). In addition, non-invasive administration of buprenorphine via voluntary ingestion has been found to improve post-operative recovery (Flecknell et al., 1999; Goldkuhl et al., 2008) and reduce stress responses for at least 18 h (Goldkuhl et al., 2008). In mice, buprenorphine is known to possess analgesic effects after parenteral administration, although effective doses and duration of action based on analgesiometric tests vary depending on test and route of administration (Flecknell, 1984; Gades et al., 2000; Christoph et al., 2005). However, studies of voluntary ingestion in mice have, to our knowledge, not been published, and very few studies into the kinetics of buprenorphine in this species have been made (Yu et al., 2006). In order to develop voluntary ingestion modes of buprenorphine as an analgesic strategy for mice, it is important to assess the post-administration serum concentration of the drug.

The present study was designed in order to investigate the concentration and duration of buprenorphine in the circulation of mice, using four different means of delivery, namely, IV injection, SC injection, oral administration through gavage (PO) and oral administration through voluntary ingestion in a hazelnut spread, Nutella (VIN). It was hypothesised that the serum concentration of buprenorphine after the oral routes of administration would be at least as high as for the parenteral administration routes.

Material and methods

Animals

Forty-eight male NMRI mice (Taconic) weighing 30–35 g were included in the study, using four delivery methods for the analgesic agent and four sampling

* Corresponding author. Address: Department of Experimental Medicine, University and University Hospital of Copenhagen, Copenhagen, Denmark. Tel.: +45 35 32 62 72; fax: +45 35 32 73 99.
E-mail address: klasab@sund.ku.dk (K.S.P. Abelson).

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windows. Upon arrival, the animals were acclimatised to the housing conditions and habituated to routine handling by animal technicians for 7 days prior to the experiment. The mice were housed in groups of 2–3 in individually ventilated polycarbonate type II cages (Techniplast). Cages were enriched with cardboard hides and with aspen chips (4H, Tapvei Oy) as bedding material. The animals were subjected to standard animal house conditions: Diurnal rhythm was regulated through a 12 h light/12 h dark cycle (lights on from 06:00 to 18:00 h), air was changed 12–14 times/h, temperature was kept at 21–22 °C and relative humidity kept at 55 ± 10%. Extruded pellets (Atromin 1319, Brogaarden) and acidified tap water (pH 3.0) were provided ad libitum.

The relevant Danish authorities (license no. 2005/561–1059 under the Danish Ministry of Justice – the Animal Experiments Inspectorate) approved the present study.

Drug administration and blood sampling

Buprenorphine (Temgesic, Schering-Plough Europe) was administered via four routes of administration as follows: IV (0.05 mg/kg bodyweight [BW]), SC (0.05 mg/kg BW), oral gavage (0.4 mg/kg BW), and through voluntary ingestion (0.4 mg/kg BW), between 08:00 and 21:00 h. The parenteral dose was based on recommendations in the literature (Dobromylskyj et al., 2000; Hedenqvist and Hellebrekers, 2003), and the oral dose on previous experiences from voluntary ingested buprenorphine in rats (Flecknell et al., 1999; Goldkuhl et al., 2008). For voluntary ingestion, buprenorphine tablets were ground in a mortar to a fine powder and mixed in 2 g/kg BW of a hazelnut spread, Nutella (Ferrero). To familiarise the mice with Nutella, animals in the voluntary ingestion group were provided with Nutella 18–24 h before being given the analgesia-spiked mixture. This ensured that the inherently neophobic animals would ingest the analgesic immediately, once provided. All animals included in the study consumed all of the buprenorphine–Nutella mixture within 15 min.

Blood was collected from IV injected mice immediately and at 2, 6 or 12 h after injection. Blood from the animals of the other groups were similarly sampled at 2, 6, 12 h or 24 h after exposure to the analgesic. The sampling frequencies were chosen based on the reported duration of the effect mentioned above. Blood was collected by submandibular venepuncture. One sample of approximately 200 μL of blood was collected from each animal per experiment with a 2 week period of restitution between experiments, i.e. two collections of 200 μL per animal. In total, six replicates were procured for each time point and administration method. No animal was subjected to the same route of administration or sampling occasion twice.

Buprenorphine quantification

Serum was separated from the blood samples and analysed in duplicates using the Buprenorphine One-step ELISA (International Diagnostic Systems) in accordance with the manufacturer's instructions. For improved accuracy the provided standards were supplemented with additional dilutions to yield a five point standard curve consisting of concentrations 0, 0.5, 1, 1.5 and 3 ng/mL. All known cross-reactivities are reported by the manufacturer at <0.06%, with the exception of norbuprenorphine, which cross-reacts at 1.1%. No analytical sensitivity was given by the manufacturer, but the analysis kit has previously been validated for samples with corticosterone concentrations as low as 0.2 ng/mL (Cirimele et al., 2004). The absorbencies were recorded at 450 nm (reference wavelength: 650 nm) using a Thermo Multiskan Ex microplate reader (Thermo Fisher Scientific).

Data treatment

The serum concentration over time of a xenobiotic can be described using compartment models in accordance to the generalised Eq. (1), assuming a first order rate of elimination.

$$C_{serum} = \sum_{i=1}^{n} C_i e^{-k_i t} \tag{1}$$

C_{serum} is the serum concentration of buprenorphine at t h past administration in a model featuring n compartments. C_i and k_i are hybrid constants of starting concentration and elimination, respectively, contributed by compartment i. The half-life of a xenobiotic is most commonly defined through the elimination in the terminal phase in order to avoid factoring in the effect of distribution. Half-lives ($t_{½}$) were thus calculated as per Eq. (2), where k_n is the elimination rate constant for the terminal phase of the concentration–time profile.

$$t_{1/2} = \frac{\ln 2}{k_n} \tag{2}$$

For easier between-methods comparison, the 24 h serum concentration, which we define as the area under curve (AUC) for the first 24 h post-administration, was calculated using the analytical solution for the integral (3) using t_{end} = 24 h. This presents the special cases for IV[1] (4) and the other routes of administration (5). For the latter we assume a linear increase in concentration, from nothing at administration, to a peak concentration before the exponential decay, described by Eq. (1), sets in. We hence make the assumptions $C_{serum}(0) = 0$ and $t_{peak} = 2$ which is our best estimate at a peak concentration.

$$A_{\mathrm{AUC}} = \int_0^{t_{end}} C_{serum}(t)dt \tag{3}$$

$$A_{\mathrm{AUC,IV}} = \int_0^{t_{end}} C_{serum}(t)dt = \sum_{i=1}^{n} \frac{C_i}{k_i}(1 - e^{-k_i t_{end}}) \tag{4}$$

$$A_{\mathrm{AUC,X}} = \int_0^{t_{end}} C_{serum}(t)dt = \frac{C_{serum}(t_{peak}) \times t_{peak}}{2} + \int_{t_{peak}}^{t_{end}} C_{serum}(t)dt$$

$$\int_{t_{peak}}^{t_{end}} C_{serum}(t)dt = \sum_{i=1}^{n} \frac{C_i}{k_i} \times {}_{t_{end}}^{t_{peak}}[e^{-k_i t}] \tag{5}$$

Two (Ohtani et al., 1994) and three (Yu et al., 2006) compartment models have been applied to great accuracy in describing the distribution and elimination of buprenorphine in rodents. When fitting models to the experimental data however a maximum of two compartments was used because of the small number of time points. Least squares fitting of compartment models to the experimental data was carried out in MATLAB v.7.8 (The Mathworks), using the curve fitting toolbox. To account for the increase in variables in the higher order compartment model – commonly producing a better fit, but not always a better model – the fitted curves were evaluated using adjusted R squared (Eq. (6)). Here $\sigma_{SS,tot}$ is the total sum of squares and $n_{df,tot}$ is its corresponding degrees of freedom. Analogously $\sigma_{SS,err}$ and $n_{df,err}$ are the residual sum of squares and degrees of freedom for the error.

$$\bar{R}^2 = 1 - \frac{\sigma_{SS,err} \times n_{df,tot}}{\sigma_{SS,tot} \times n_{df,err}} \tag{6}$$

Other kinetic parameters derived from data are the bioavailability (7), the mean residence time (8), the rate of clearance (11) and the apparent total volume at steady state (12). For the mean residence time we first calculated the area under the first moment curve (Eqs. (9) and (10)). Indices IV and X, used throughout, indicate IV administration and administration route 'X', respectively (i.e. SC, PO or VIN). D represents the administered dose.

$$F_{\mathrm{X}} = \frac{D_{\mathrm{IV}} \times A_{\mathrm{AUC,X}}}{D_{\mathrm{X}} \times A_{\mathrm{AUC,IV}}} \tag{7}$$

$$t_{\mathrm{MRT,X}} = \frac{A_{\mathrm{AUMC,X}}}{A_{\mathrm{AUC,X}}} \tag{8}$$

$$A_{\mathrm{AUMC,IV}} = \int_0^{t_{end}} t \times C_{serum}(t)dt = \sum_{i=1}^{n} C_i \int_0^{t_{end}} t \times e^{-k_i t}dt$$

$$= \sum_{i=1}^{n} \frac{C_i}{k_i^2} - \frac{C_i \times e^{-k_i t_{end}}}{k_i} \times \left(t_{end} + \frac{1}{k_i}\right) \tag{9}$$

$$A_{\mathrm{AUMC,X}} = \frac{C_{serum}(t_{peak}) \times t_{peak}^2}{2} + \int_{t_{peak}}^{t_{end}} t \times C_{serum}(t)dt$$

$$\int_{t_{peak}}^{t_{end}} t \times C_{serum}(t)dt = \sum_{i=1}^{n} \frac{C_i}{k_i^2} \times {}_{t_{end}}^{t_{peak}}\left[(k_i t + 1) \times e^{-k_i t}\right] \tag{10}$$

$$r_{\mathrm{CL}} = \frac{D_{\mathrm{IV}}}{A_{\mathrm{AUC,IV}}} \tag{11}$$

$$V_{\mathrm{SS}} = r_{\mathrm{CL}} \times t_{\mathrm{MRT,IV}} \tag{12}$$

Results

A best regression fit was achieved by applying a two compartment model to the parenteral modes of administration (IV, SC), whereas a single compartment model best described the oral modes of delivery (PO, VIN) (Fig. 1). Calculated regression constants, goodness of fit, 24 h serum concentrations, and estimated kinetic constants are summarised in Table 1. Forcing a two compartment model onto the data from the oral modes of delivery resulted in a negative adjusted R^2.

[1] Extending this integral towards infinity yields the following, more commonplace, equation for the AUC:

$$\lim_{t_{end} \to \infty} A_{\mathrm{AUC}} = \sum_{i=1}^{n} \frac{C_i}{k_i}$$