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Clinical efficacy of buprenorphine after oral dosing in rats undergoing major surgery

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Abstract

Serum corticosterone, serum buprenorphine, body weight change, consumption of food and water and behaviour-based pain assessment were measured in catheterised and non-catheterised male Wistar rats undergoing myocardial infarct (MI) surgery under general anaesthesia following buprenorphine dosing by subcutaneous (Bup-SC, 0.05 mg/kg) and oral (Bup-O, 0.4 mg/kg) routes. Buprenorphine was dosed subcutaneously at half an hour before and 8, 16 and 24 hours after surgery (Bup-SC), orally at one hour before surgery (Bup-O1) or at one hour before and 12 hours after surgery (Bup-O2) in catheterised rats and at one hour before and 24 hours after surgery (Bup-O24) in non-catheterised rats. Serum corticosterone, body weight changes and food and water consumption were not significantly different between treatments in catheterised rats. Bup-SC resulted in rapidly decreasing serum concentrations below the clinically effective concentrations (1 ng/mL) already at two hours after the first dose. Bup-O provided significantly higher and slowly decreasing serum concentrations, at or above clinically effective concentrations, for 24 hours (Bup-O1) and 42 hours (Bup-O2) after surgery. In non-catheterised rats, body weight development and food consumption were significantly higher in Bup-O24 rats compared to Bup-SC rats. The results indicate that a SC buprenorphine dose of 0.05 mg/kg every eight hours provides long periods of serum concentrations below clinically effective levels, and that a higher dose and/or more frequent dosage are required to provide stable serum concentrations at or above clinically effective levels. A single oral buprenorphine dose of 0.4 mg/kg provides clinically effective and stable serum concentrations for 24 hours in rats after MI surgery.

Keywords

Buprenorphine, corticosterone, analgesia, oral gavage, myocardial infarct

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Introduction

Effective analgesia is of vital importance in surgical animal models for scientific, ethical and legal reasons. In addition to being efficacious, practical aspects of dosage and animal stress during dosing are important considerations for choice of analgesia protocol. Slow-release formulations of buprenorphine (Bup), such as Buprenorphine HCl SR,[1–3] are widely used in rodents where available, as they provide consistent Bup serum concentrations above the assumed clinically effective concentration of 1 ng/mL in rodents. However, these products are currently not universally available, and repeated subcutaneous (SC) dosage of Bup remains

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the best option for many surgical procedures in rodents, requiring repeated injections and transient physical restraint during both daylight and night cycles. The recommended Bup-SC dosage after surgery is every four to six hours in mice and every six to eight hours in rats.[4,5] Others have documented that the duration of clinically effective serum concentrations after SC dosing of Bup is likely no more than about four hours in mice (0.1 mg/kg)[6] and less than two hours in rats (0.05 mg/kg).[7]

Continuous oral administration of Bup (Bup-O) via dietary pellets with Bup after abdominal surgery of moderate severity has been described in mice.[8] Following habituation and single housing, oral administration of Bup through voluntary ingestion of a dietary treat prior to surgery has been shown to provide clinically effective plasma levels of Bup in rats for about 14–18 hours during minor surgical procedures, such as jugular vein catheterisation,[7] and the authors speculate about the feasibility of using Bup-O by voluntary ingestion for other and more invasive procedures.

The aims of the study were to evaluate serum corticosterone and Bup kinetics during and after Bup-SC and Bup-O in rats and to evaluate the clinical effects after myocardial infarct (MI) surgery in general anaesthesia. Our hypothesis was that oral dosing of Bup would be non-inferior to or better than the traditional regimes of SC Bup dosing used currently, providing adequate analgesia after surgery in rats.

Methods

Ethical statement

The animal experiments described in this paper were approved by the Norwegian Food Safety Authority (FOTS ID 15889) and conform to the principles and requirements of the European Convention for the Protection of Vertebrate Animals (ETS 123), and EU directive 2010/63/EU.

Animals

Eight- to 10-week-old male Wistar (WI) rats (RjHan: WI), delivered as SPF animals by Janvier Labs (Le Genest Saint Isle, France), were used in these studies. Sixteen rats with a mean ± SD body weight of 308 ± 17 g were used in the pilot study. Forty-eight rats with a mean ± SD body weight of 326 ± 16 g were used in study A. Forty rats with a mean ± SD body weight of 294 ± 14 g were used in study B. In order to exclude hormonal fluctuations caused by the oestrus cycle as a confounding variable, only males were included.

Study design

Pilot study. The purpose of the pilot study was to investigate the time course of return to baseline serum corticosterone concentrations and circadian rhythm after catheterisation surgery. Importantly, normalisation of these parameters reduced the risk that prior surgery influenced the effect estimations during the main surgical procedure (i.e. MI). To avoid influencing the clinical effects and measurements of Bup in study A, only local anaesthesia (bupivacaine) was used during catheterisation in the pilot study and study A. Catheterisation was performed between 06:50 and 09:36, followed by connection to an Accusampler sampling robot (AS; Dilab, Lund, Sweden) immediately after surgery. Blood was sampled automatically from the catheterised animals by means of the AS, reducing or eliminating artefactual effects on corticosterone levels due to operator presence and physical restraint during blood sampling. Blood was sampled for 70 hours after catheterisation to document normalisation of serum corticosterone levels and circadian rhythm. Three rats included in the pilot study and two data points were excluded due to surgical complications and blocked catheter/robot malfunction, respectively.

Study A. Based on normalisation of corticosterone levels and circadian rhythm from day 1 after catheterisation (pilot study), animals catheterised on day 0 were dosed with Bup and subjected to MI surgery on day 2 after catheterisation. After block randomisation to treatment groups, surgery 1 (catheterisation) was performed between 07:00 and 09:20 on day 0, as described for the pilot study. The patency of the catheter was maintained for two days after surgery by continuous connection to the AS. Surgery 2 (MI surgery) was performed between 07:01 and 08:35 on day 2. After preemptive dosing with Bup, the room housing the animals was left undisturbed until immediately before the start of the MI surgery. Animals in group Bup-SC were dosed SC with 0.05 mg/kg bw of Bup (Temgesic injection, 0.3 mg/mL; Indivior Europe Ltd, Dublin, Ireland), 44 ± 7 minutes before the start of the MI surgery. Animals in groups Bup-O1 and Bup-O2 were dosed by oral gavage with 0.4 mg/kg of a suspension of Bup tablets (Temgesic sublingual tablet, 0.4 mg; Indivior Europe Ltd) in tap water[7,9,10] at 70 ± 11 and 70 ± 5 minutes before the start of the MI surgery, respectively. Serum corticosterone and Bup kinetics were measured for 46 hours after MI surgery. Ten rats in study A were excluded due to cardiac arrest or surgical or technical complications. For details, see the Supplemental Material.

Study B. Based on the sustained levels of serum Bup at clinically effective levels in study A, the clinical effects of Bup-SC (dosed as described in study A) or two oral doses of 0.4 mg/kg Bup at one hour before and 24 hours after surgery (Bup-O24) were evaluated during routine MI surgery of rats used in another research project. After block randomisation to treatment groups, pre-emptive dosing of groups Bup-SC and Bup-O24 was done at 35 ± 9 and 63 ± 17 minutes before the start of MI surgery, respectively. Surgeries in study B (MI surgery) were performed between 08:06 and 14:09 on day 0. The clinical effects were evaluated by body weight changes, consumption of food and water, and behaviour-based pain assessment, as previously described by Jirkof et al.[6] Twenty-four rats in study B were excluded due to cardiac arrest. For details about animal exclusion and behaviour-based pain assessment, see the Supplemental Material. Group sizes of 12–16 rats in the pilot study and study A were estimated as a suitable minimum based on the authors experience with the MI model and literature reports of serum corticosterone measurements in catheterised rats,[7,9] and power analysis was not performed. In study B, the number of rats included in the clinical trial reflects the number of rats routinely included during MI surgery at our institution.

Husbandry and inclusion/exclusion criteria

For details about housing, husbandry, health monitoring and inclusion/exclusion criteria, see the Supplemental Material.

Preoperative and surgical procedures

General anaesthesia with isoflurane in oxygen carrier gas was induced in an induction chamber (5%) and maintained at 2.5%–3% isoflurane to effect on a coaxial mask during preoperative procedures and catheterisation of the carotid artery and a VentElite ventilator (Harvard Apparatus, Holliston, MA) during MI surgery. A dose of 0.6 mg Bupivacain (Marcain 2.5 mg/mL; Aspen Nordic, Ballerup, Denmark) was injected SC on either side of the incision sites at least three minutes before the start of surgery.[11] For details about the preoperative and surgical procedures in the pilot study and studies A and B and for details about the oral Bup suspension, see the Supplemental Material.

Blood sampling and postoperative procedures

Pilot study. The first blood sample (at five minutes) started on average eight minutes after the end of surgery. Blood samples (150 µL) were obtained at five minutes and then at 2, 6, 10, 14, 18, 24, 36, 48, 60 and 70 hours after starting the AS. Following the last blood sample on day 3, the animals were weighed with harness and then euthanised by CO_2 asphyxiation.

Study A. Baseline blood samples obtained on day 2, at approximately 46 hours after surgery 1 and one and a half hours before MI surgery, were taken immediately before pre-emptive dosing of Bup. The first blood sample (at five minutes) started on average eight minutes after the end of MI surgery. Following MI surgery, blood samples (150 µL) were obtained at five minutes and then at 2, 6, 10, 14, 18, 24, 30, 36, 42 and 46 hours after starting the AS. Following the last blood sample at 46 hours on day 4, the animals were weighed with harness and then euthanised by CO_2 asphyxiation.

Study B. MI surgery of the animals in group B was performed in relation to another project. The animals were euthanised by exsanguination followed by harvest of the cardiac tissues during deep terminal isoflurane anaesthesia.

For details about AS blood sampling and catheter maintenance in the pilot study and study A, actual time of post-operative Bup dosing in studies A and B and details related to CO_2 asphyxiation, see the Supplemental Material.

Quantification of corticosterone and Bup

Serum corticosterone was analysed with a quantitative Corticosterone ELISA Kit (Enzo Life Sciences, Inc., Farmingdale, NY) according to the manufacturer's instructions. Quantitative analysis of serum Bup was performed with a Buprenorphine ELISA Kit (Neogen Corporation, Lexington, KY), in principle as described by Goldkuhl et al.[7] The kit accuracy was validated by liquid chromatography–tandem mass spectrometry (LCMS/MS), and low intra- and inter-assay coefficients of variation were demonstrated. For details about blood and serum handling and corticosterone and Bup analysis, see the Supplemental Material.

Statistical analysis

Normal distribution and homogeneity of variances were tested by Shapiro–Wilk and Levene's tests, respectively. Multiple groups were compared by Welch analysis of variance (ANOVA) followed by Games–Howell's post-hoc test or Kruskal–Wallis one-way ANOVA followed by Bonferroni's post-hoc test as appropriate. Two groups were compared by Mann–Whitney *U*-test or *t*-test as appropriate. In study A, animals in groups Bup-O1 and Bup-O2 were exposed to the same surgery, handling and analgesia procedures from one and a half hours before to 10 hours after

MI surgery, and mean serum corticosterone and serum Bup values were not significantly different at any of these time points. Serum corticosterone and Bup data from Bup-O1 and Bup-O2 were hence combined into a pooled Bup-O12 group for statistical comparison with Bup-SC at these time points. Composite scores were used for statistical analysis of behaviour-based pain assessment. IBM SPSS Statistics for Windows v27 (IBM Corp., Armonk, NY) was used for statistical analysis, and p-values <0.05 were considered statistically significant.

Results

None of the animals included in the pilot study or study A and B displayed abnormal behaviour indicative of stress or pain that warranted premature termination according to pre-determined humane end points. No clinical signs of pica behaviour, hyperactivity, sedation or respiratory depression were observed in any of the animals in studies A and B.

Pilot study

Following peak corticosterone levels at the first sampling after surgery (five minutes), corticosterone levels decreased markedly at two hours and returned to normal circadian rhythm from 24 hours onwards (Figure 1). Based on these results, catheterisation (surgery 1) and MI surgery (surgery 2) were performed on days 0 and 2, respectively, in study A.

Study A

As in the pilot study, peak corticosterone levels were observed at five minutes after surgery, followed by a marked decrease at two hours (Figure 2). No significant differences among any of the groups were observed at any time point after MI surgery. A clear circadian rhythm, with difference in day- and nighttime corticosterone levels, was not readily apparent in study A.

The distribution of serum Bup levels in the pooled Bup-O12 group was significantly different from that of the Bup-SC group at five minutes, two hours and six hours after surgery (Figure 3). The serum Bup levels of the Bup-O1 group were significantly higher than those of the Bup-SC group at 14 hours. The serum Bup levels of the Bup-O2 group were significantly higher than those of the Bup-SC group at all time points and higher than the Bup-O1 group at the 14- and 24-hour time points.

While serum Bup levels peaked at five minutes after surgery in Bup-SC (1.3 ± 0.2 ng/mL), levels continued to rise until two hours after surgery in the Bup-O12 group (2.6 ± 0.5 ng/mL), indicating prolonged absorption and/or enterohepatic recirculation. Following the repeated oral Bup dose at 12 hours in the BupO2 group, serum Bup peaked at 5.1 ± 0.5 ng/mL. In the Bup-O1 and Bup-O2 groups, mean serum Bup remained relatively stable at or above clinically

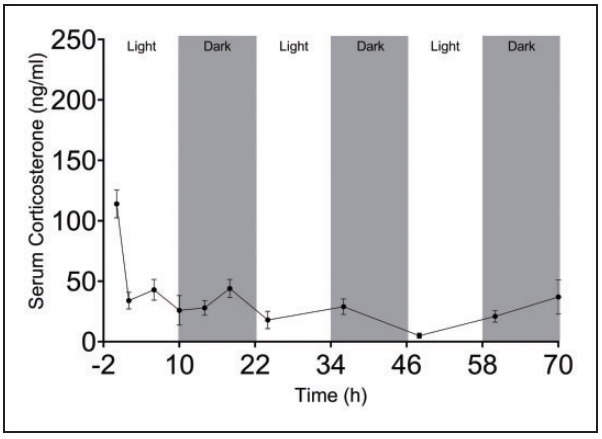

Figure 1. Baseline serum corticosterone levels during automated blood sampling of freely moving tethered rats in the pilot study from five minutes to 70 hours after catheterisation of the carotid artery. Mean ± standard error of the mean (SEM), 11–13 samples per time point. The shadowed boxes indicate the periods of darkness.

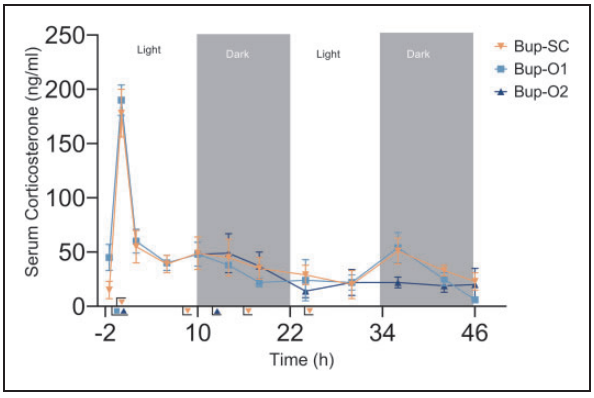

Figure 2. Serum corticosterone levels obtained by automated blood sampling from the carotid artery of freely moving tethered rats in study A from one and a half hours before to 46 hours after myocardial infarct (MI) surgery. Mean ± SEM, 8–14 samples per time point per analgesia group. Data analysed by Mann–Whitney *U*-test from one and a half hours before to 10 hours after surgery (when Bup-O1 and Bup-O2 data were pooled) and with Kruskal–Wallis one-way analysis of variance (ANOVA). The shadowed boxes indicate the periods of darkness. Bup-SC: subcutaneous (SC) dosing of Temgesic injection, 0.05 mg/kg at 30 minutes before and 8, 16 and 24 hours after surgery. Bup-O1: oral gavage with Temgesic sublingual tablet suspension, 0.4 mg/kg at one hour before surgery. Bup-O2: oral gavage with Temgesic sublingual tablet suspension, 0.4 mg/kg at one hour before and 12 hours after surgery. Buprenorphine dosing indicated below *x*-axis, with symbols corresponding to the figure legend.

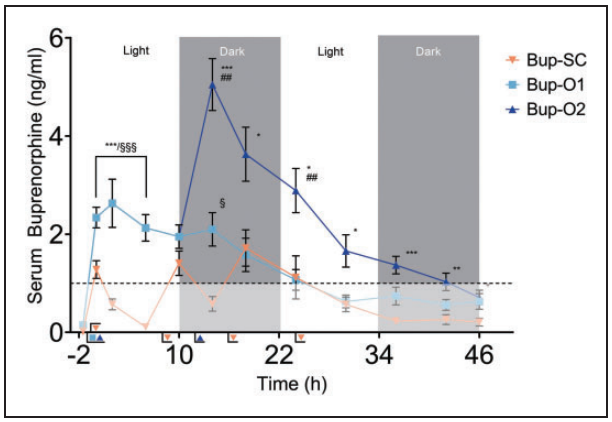

Figure 3. Serum buprenorphine levels obtained by automated blood sampling from the carotid artery of freely moving tethered rats in study A from an hour and a half before to 46 hours after MI surgery. Mean ± SEM, 8–14 samples per time point per analgesia group. Dashed line indicates clinical target concentration (1 ng/mL). Data analysed by Mann–Whitney *U*-test from one and a half hours before to 10 hours after surgery (when Bup-O1 and Bup-O2 data were pooled) and with Welch ANOVA with Games–Howell post-hoc test from 14 to 46 hours after surgery. Group Bup-O1 and Bup-O2 data combined during the period one and a half hours before surgery to 10 hours after surgery. § and §§§ indicate significant differences ($p < 0.05$ and $p < 0.001$, respectively) between Bup-O1 and Bup-SC at the indicated time points. *,** and *** indicate significant differences ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively) between Bup-O2 and Bup-SC at the indicated time points. ## indicates significant differences ($p < 0.01$) between Bup-O1 and Bup-O2 at the indicated time points. Shadowed boxes, legends on Bup-SC, Bup-O1, Bup-O2 and time of Buprenorphine dosing as described in Figure 2.

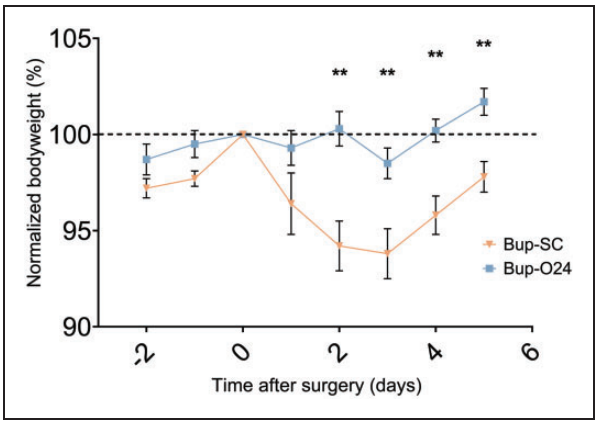

Figure 4. Body weight development, relative to body weight at day 0, in non-catheterised rats from two days before to five days after MI surgery in study B. Mean ± SEM, 6 and 10 samples per analgesia group. Data analysed by *t*-test. ** indicate significant differences ($p < 0.01$) between Bup-O24 and Bup-SC at the indicated time points. Bup-SC: SC dosing of Temgesic injection, 0.05 mg/kg at 30 minutes before and 8, 16 and 24 hours after surgery. Bup-O24: oral gavage with Temgesic sublingual tablet suspension, 0.4 mg/kg at one hour before and 24 hours after surgery.

effective concentrations of 1 ng/mL for 24 and 42 hours after surgery, respectively. Following SC administration, serum Bup levels rapidly decreased after the first and second dose. The concentrations at two and six hours after the first dose (0.6 ± 0.1 and 0.1 ± 0.2 ng/mL, respectively) indicate that the duration of clinically effective serum concentrations is around two hours. Following the third dose at 16 hours, serum concentrations of Bup persisted longer, being >1 ng/mL at 18 and 24 hours and 0.57 ± 0.15 ng/mL at 30 hours after surgery. Except for the 10-hour time point (two hours after the second SC dose), the Bup concentrations after SC dosing were consistently lower during the first 18 hours after surgery than observed in the oral dosing groups.

Compared with their baseline weight at MI surgery (day 2), all animals in the Bup-SC group lost weight, while 2 of 14 and 3 of 11 animals in the Bup-O1 and Bup-O2 groups, respectively, had gained weight by day 4. However, the differences in mean body weight change and the intake of food and water for 46 hours after MI surgery were not statistically significantly different between groups (data not shown).

Study B

Mean body weight, normalised to body weight at time of MI surgery (day 0), was significantly lower in the Bup-SC group at days 2–5 after surgery (Figure 4). The mean body weight development from day 0 to day 2 after surgery in the Bup-O24 group ($0.3\% \pm 0.9\%$) was significantly higher than in the Bup-SC group ($-5.8\% \pm 1.3\%$).

The body weight change in the Bup-SC group was reflected in significantly reduced food consumption from day 0 to day 2, whilst the water consumption was not significantly different between the groups (Figure 5).

The distribution of composite scores of behaviour-based pain assessment was not significantly different between dosing groups (Figure 6). The mean scores of all behaviour-based pain assessment parameters in the Bup-SC and Bup-O24 groups are illustrated in Supplemental Figure S1.

Discussion

The MI rat model is a widely used animal model to study congestive heart failure, and Bup-SC, before and every eight hours after MI surgery, has been the routine protocol used in this model at our institution, representing a commonly used analgesia protocol in rats.[12] In this study, we

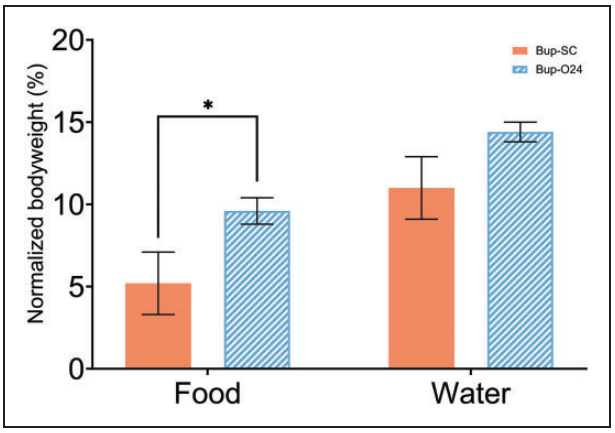

Figure 5. Food and water consumption in non-catheterised rats, relative to body weight at day 0, during the first two days after MI surgery in study B. Mean ± SEM, 6 and 10 samples per analgesia group. Data analysed by *t*-test. * indicates significant differences ($p < 0.05$) between Bup-O24 and Bup-SC. Route, time and frequency of Buprenorphine dosing as described in Figure 4.

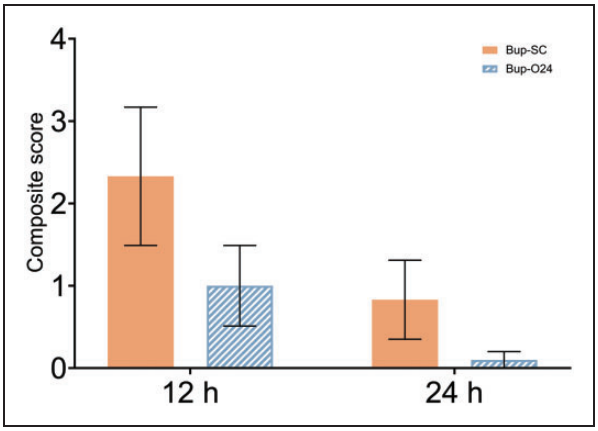

Figure 6. Composite score of behaviour-based pain assessment in non-catheterised rats at 12 and 24 hours after MI surgery in study B. Mean ± SEM, 6 and 10 samples per analgesia group. Data analysed by Mann–Whitney *U*-test. Route, time and frequency of buprenorphine dosing as described in Figure 4.

measured serum corticosterone and Bup in catheterised rats undergoing MI surgery, and evaluated clinical effects in non-catheterised rats after routine MI surgery. We demonstrate that a single oral dose of Bup (Bup-PO) provides consistent therapeutic levels of circulating drug for more than 24 hours, superior to that of Bup administered SC three times in the same period (Bup-SC).

A target serum Bup concentration of 0.5–0.7 ng/mL is required for effective analgesia in humans,[13] while effective concentrations in rodents are assumed to be ≥1 ng/mL.[14] Bup is characterised by a relatively slow distribution to target sites, as well as a relatively slow association/dissociation with μ opioid receptors in target tissues, despite rapid increase in serum concentrations.[14] This hysteresis between pharmacokinetics (serum concentrations) and pharmacodynamics (effects of Bup) may provide a longer duration of action than indicated by the serum concentration. In the present study A, where 0.05 mg/kg Bup was dosed SC 30 minutes before MI surgery and every eight hours for 24 hours after surgery, we demonstrate clinically effective serum concentrations for about two hours or less after each of the first two doses, comparable to previous reports.[7] Serum kinetics that are compatible with an eight-hour dosage were not observed until after the third dose at 16 hours after surgery.

The mean serum concentrations of Bup were >1 ng/mL for up to 24 hours in the single oral dose group (Bup-O1), and up to 42 hours in the oral dosing group that was re-dosed at 12 hours after surgery (Bup-O2). Although the significant differences in serum concentrations likely reflect differences in dose in the SC and oral groups, the marked difference in the decay of serum levels likely reflects prolonged absorption, and possibly enterohepatic recirculation, after oral dosing. With more stable serum concentrations of Bup ≥1 ng/mL for ≥24 hours, oral dosing probably results in more sustained and superior analgesic effect, and any effects of hysteresis will hence only manifest at the start and end of this relatively long duration of action. Despite peak serum Bup concentrations >2 and 5 ng/mL in the Bup-O1 and Bup-O2 groups, respectively, no adverse clinical signs attributable to the high Bup concentrations were observed. These observations are comparable to results reported by Foley et al. after dosing of sustained-release formulation of Bup in rats, where clinical evidence of analgesia and absence of adverse effects on eating, drinking, activity and pica behaviour was observed for three days after dosing.[2] Foley et al.[2] speculate that the absence of adverse effects is associated with stable serum concentrations (around 1 ng/mL from 24 to 72 hours, with an initial peak around 3 ng/mL at four hours after dosing), as opposed to the variable serum concentration observed after SC dosing of Bup.

Despite the marked difference in duration of clinically effective Bup serum concentrations in the Bup-SC, Bup-O1 and Bup-O2 groups, no significant differences in serum corticosterone, body weight change and intake of food or water were observed after MI surgery in study A. This suggests that neither of the two oral Bup dosing regimens was inferior to the SC dosing regimen following MI surgery in catheterised rats. However, the lack of differences may also indicate the limitations of corticosterone, body weight and food/water intake measurements as indicators of pain and distress when animals are connected via

a catheter to the AS for several days in study A. Based on these limitations, and the observed serum kinetics that indicate a duration of action of 24 hours after oral dosing, we evaluated the clinical effects of oral dosing before and 24 hours after MI surgery in rats that were not catheterised.

In study B, where Bup was dosed by oral gavage at one hour before and 24 hours after MI surgery, the body weight development and intake of diet after MI surgery indicate more effective analgesia after oral dosing. This is likely caused by more stable serum concentrations of Bup, at or above the clinically effective threshold, for two days after surgery.

The comparable levels of serum Bup recorded after SC dosing suggests that the analytical accuracy in the present study is comparable to previous reports.[7] In the present study, the five-minute blood samples were obtained approximately 25–40 minutes earlier after dosing than described by Goldkuhl et al.[7] Lower serum Bup concentrations than reported by Goldkuhl et al.[7] were hence expected in study A (due to prolonged but slower intestinal absorption). However, the mean serum Bup levels observed in study A were about double that seen by Goldkuhl et al. after surgery in the voluntary ingestion group (about 0.8 and 1.3 ng/mL at five minutes and two hours, respectively). The higher serum levels in the oral dosing groups (both in absolute concentrations and relative to the SC dosing group) reflect a significantly higher dose but may also suggest a more rapid and perhaps more complete uptake of the oral Bup dose when solubilised in tap water and dosed by oral gavage as compared to voluntary ingestion of the same dose of Bup in Nutella.[7] Oral gavage was used in the present study, as we were not able to secure voluntary ingestion of Bup in Nutella at a predictable time and dose in postoperative animals. We did not encounter any adverse effects from oral gavage and did not observe a level of stress and discomfort as being different from the transient stress experienced by the rats during SC injection.

Our study has several limitations. LCMS/MS is the gold standard for chemical analysis of samples, providing a high accuracy and molecular specificity, higher than analysis of individual samples by antibody-based analytical techniques.[15] Measurements of corticosterone and Bup were done with the enzyme-linked immunosorbent assay (ELISA) technique, and cross-reactivity and binding characteristics of antigens affect the accuracy and specificity of analysis with this ELISA technique. The results from the present study, particularly absolute concentrations, must hence be interpreted with caution. However, the analysis of Bup calibrator samples by ELISA and LCMS/MS demonstrates an acceptable bias of Bup quantification by ELISA in the present study. In addition, the intra- and inter-assay coefficients of variance indicate an acceptable variability of Bup analysis by the ELISA technique. In the present study, up to 12 serum samples of approximately 70–80 μL volume per animal were used for duplicate analysis of both corticosterone and Bup. Given the limitations of total blood sample volume that can be obtained from rodents, and the relatively high minimum sample volume required for LCMS/MS analysis, the modified ELISA kit provides an analytical method that is well suited for assessing dynamic differences in serum Bup concentrations at multiple discrete time points in rodents. The ability by the modified ELISA kit to provide a higher time resolution of the serum kinetics in rodents must obviously be considered and balanced by said limitations in accuracy of absolute values.

We have used serum corticosterone as an indicator of stress and a surrogate indicator for pain after MI surgery. While the corticosterone data indicate non-inferiority of oral versus SC dosing of Bup, the apparent normalisation in serum corticosterone from two hours after major surgery suggests the limitations in serum corticosterone as an indicator of pain and certainly as a method to discriminate real differences between the Bup dosing groups in study A. Consequently, we chose to evaluate oral versus SC dosing of Bup by clinical observations and behaviour-based pain assessment in study B, despite inherent methodological limitations with these techniques. Spillage of food and water may compromise calculations of diet and water consumption in conventional cages, and subjective evaluation may affect behaviour-based pain assessment, even with all efforts to standardise evaluations and despite blinding of the evaluator.

We planned for voluntary ingestion of Bup in Nutella. However, despite rapid habituation of naïve animals, predictable voluntary oral ingestion of Nutella (with or without Bup) was not feasible at day 2 after catheterisation surgery, as the animals did not reliably consume Nutella in our study. To ensure accuracy in time of dosing, a suspension of Temgesic sublingual tablet was dosed by oral gavage, in accordance with previously reported use of this Bup formulation for voluntary ingestion.[7,9,10,16] With a Bup concentration of 0.6 mg/mL in the oral dosing solution, a maximum Bup hydrochloride solubility of 17 mg/mL in water[17] and rapid dissolution in lukewarm water, we observed the Temgesic sublingual tablet to be both practical and effective in use.

The mortality rates after MI surgery were higher than what is to be expected after MI surgery in rats, despite being performed by a surgeon with specialised training in rodent surgery and extensive prior experience with MI surgery. Compared to prior experience with the MI model in rats, we experienced an increase in

mortality rates after surgery in the period around this study. Indeed, we undertook a blinded trial of rats from several vendors and found that rats from the vendor utilised in this study have excessive mortality during MI surgery (unpublished observations). Consequently, we have now moved to a different animal vendor. Although the animals used in this study had a higher rate of postoperative mortality, we believe the findings regarding analgesic efficacy retain their validity.

Only male RjHan:WI rats were used in these studies. Future studies, including female rats of relevant stocks and strains, should be performed to evaluate the generalisability of the serum kinetics and clinical efficacy of the analgesia protocols in the present study. In this context, other dose levels and dosages by both SC and the oral route should be investigated.

In summary, our results indicate that a single oral Bup dose of 0.4 mg/kg provides stable and clinically effective serum concentrations for 24 hours in rats, and likely effective analgesia for at least the same duration. This assumption is supported by the clinical results observed in rats dosed with oral Bup at one hour before and 24 hours after MI surgery. Furthermore, a SC Bup dose of 0.05 mg/kg every eight hours provides clinically effective serum concentration for two hours or less, and likely considerably less than eight hours duration of action, after each of the two first dosages. When Bup is dosed by the SC route, a higher dose and/or dosage are probably required to avoid long periods of insufficient serum concentrations during the first 16 hours after surgery in rats.

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Supplemental material

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Efficacité clinique de la buprénorphine après administration orale chez les rats subissant une intervention chirurgicale majeure

Résumé

La corticostérone sérique, la buprénorphine sérique, le changement de poids corporel, la consommation de nourriture et d'eau et l'évaluation de la douleur basée sur le comportement ont été mesurés chez des rats Wistar mâles cathetérisés et non catheterisés subissant une intervention chirurgicale pour un infarctus du myocarde (IM) sous anesthésie générale, après administration de buprénorphine par voie sous-cutanée (Bup-SC, 0,05 mg/kg) et orale (Bup-O, 0,4 mg/kg). La buprénorphine a été dosée par voie sous-cutanée à 1 demie-heure avant l'intervention et à 8, 16 et 24 heures après l'intervention chirurgicale (Bup-SC), par voie orale à 1 heure avant l'intervention chirurgicale (Bup-O1), ou à 1 heure avant et à 12 heures après l'intervention chirurgicale (Bup-O2) chez les rats cathétérisés, et à 1 heure avant et 24 heures après l'intervention chirurgicale (Bup-O24) chez les rats non cathétérisés. La corticostérone sérique, les changements de poids corporel et la consommation d'eau et de nourriture n'étaient pas significativement différents entre les traitements chez les rats cathétérisés. La BUP-SC entraînait une diminution rapide des concentrations sériques, inférieures aux concentrations cliniquement efficaces (1 ng/ml) déjà à 2 heures après la première dose. La BUP-O fournissait des concentrations sériques significativement plus élevées et en diminution lente, à des concentrations cliniquement efficaces ou supérieures, pendant 24 heures (Bup-O1) et 42 heures (Bup-O2) après l'intervention chirurgicale. Chez les rats non cathétérisés, le développement du poids corporel et la consommation alimentaire étaient significativement plus élevés chez les rats BupO24 que chez les rats Bup-SC. Les résultats indiquent qu'une dose de buprénorphine SC de 0,05 mg/kg toutes les 8 heures fournit de longues périodes de concentrations sériques en dessous des niveaux cliniquement efficaces, et qu'une dose plus élevée et/ou un dosage plus fréquent sont nécessaires pour fournir des concentrations sériques stables à des niveaux cliniquement efficaces ou au-dessus. Une seule dose orale de buprénorphine de 0,4 mg/kg fournit des concentrations sériques cliniquement efficaces et stables pendant 24 heures chez les rats après une chirurgie pour un IM.

Klinische Wirksamkeit von Buprenorphin nach oraler Verabreichung bei Ratten, die einem großen chirurgischen Eingriff unterzogen werden

Abstract

Bei katheterisierten und nicht katheterisierten männlichen Wistar-Ratten, die in Vollnarkose einer Myokardinfarkt(MI)-Operation unterzogen wurden, wurden nach subkutaner (Bup-SC, 0,05 mg/kg) und oraler (Bup-O, 0,4 mg/kg) Verabreichung von Buprenorphin Serumkortikosteron, Serumbuprenorphin, Veränderung des Körpergewichts, Futter- und Wasseraufnahme sowie verhaltensbasierte Schmerzbewertung gemessen. Buprenorphin wurde subkutan ½ Stunde vor und 8, 16 und 24 Stunden nach der Operation (Bup-SC), oral 1 Stunde vor der Operation (Bup-O1) oder 1 Stunde vor und 12 Stunden nach der Operation (Bup-O2) bei katheterisierten Ratten und 1 Stunde vor und 24 Stunden nach der Operation (Bup-O24) bei nicht katheterisierten Ratten verabreicht. Serumkortikosteron, Körpergewichtsveränderungen sowie Futter- und Wasseraufnahme unterschieden sich bei katheterisierten Ratten nicht signifikant zwischen den Behandlungen. Bup-SC führte zu rasch sinkenden Serumkonzentrationen, die bereits 2 Stunden nach der ersten Dosis unter den klinisch wirksamen Konzentrationen (1 ng/ml) lagen. Bup-O führte zu signifikant höheren und langsam abnehmenden Serumkonzentrationen, die 24 Stunden (Bup-O1) und 42 Stunden (Bup-O2) nach dem Eingriff bei oder über den klinisch wirksamen Konzentrationen lagen. Bei nicht katheterisierten Ratten waren die Entwicklung des Körpergewichts und die Nahrungsaufnahme bei BupO24-Ratten im Vergleich zu Bup-SC-Ratten signifikant höher. Die Ergebnisse deuten darauf hin, dass eine SC-Buprenorphin-Dosis von 0,05 mg/kg alle 8 Stunden über lange Zeiträume zu Serumkonzentrationen führt, die unter den klinisch wirksamen Werten liegen, und dass eine höhere Dosis und/oder häufigere Verabreichung erforderlich sind, um stabile Serumkonzentrationen auf oder über den klinisch wirksamen Werten zu erreichen. Eine einmalige orale Buprenorphin-Dosis von 0,4 mg/kg liefert bei Ratten nach einem MI-Eingriff klinisch wirksame und stabile Serumkonzentrationen für 24 Stunden.

Eficacia clínica de la buprenorfina tras una dosificación oral en ratas sometidas a una cirugía significativa

Resumen

Se midieron la corticosterona sérica, la buprenorfina sérica, el cambio de peso corporal, el consumo de alimentos y agua y la evaluación del dolor en base al comportamiento en ratas Wistar macho cateterizadas y no cateterizadas sometidas a cirugía de infarto de miocardio (IM) con anestesia general, tras la dosificación de buprenorfina por vía subcutánea (Bup-SC, 0,05 mg/kg) y oral (Bup-O, 0,4 mg/kg). La buprenorfina se dosificó por vía subcutánea media hora antes y 8, 16 y 24 horas después de la cirugía (Bup-SC), por vía oral 1 hora antes de la cirugía (Bup-O1), o 1 hora antes y 12 horas después de la cirugía (Bup-O2) en ratas cateterizadas, y 1 hora antes y 24 horas después de la cirugía (Bup-O24) en ratas no cateterizadas. La corticosterona sérica, los cambios de peso corporal y el consumo de agua y comida no fueron significativamente diferentes entre los tratamientos en las ratas cateterizadas. El Bup-SC dio lugar a concentraciones séricas en rápido descenso, por debajo de las concentraciones clínicamente eficaces (1 ng/ml) ya a las 2 horas de la primera dosis. El Bup-O proporcionó concentraciones séricas significativamente más altas y en lento descenso, en o por encima de las concentraciones clínicamente eficaces, durante 24 horas (Bup-O1) y 42 horas (Bup-O2) después de la cirugía. En las ratas no cateterizadas, el desarrollo del peso corporal y el consumo de alimentos fueron significativamente mayor en las ratas BupO24 en comparación con las ratas Bup-SC. Los resultados indican que una dosis de buprenorfina SC de 0,05 mg/kg cada 8 horas ofrece largos periodos de concentraciones séricas por debajo de los niveles clínicamente eficaces, y que se requiere una dosis más alta y/o una dosificación más frecuente para conseguir concentraciones séricas estables en los niveles clínicamente eficaces o por encima de ellos. Una dosis oral única de buprenorfina de 0,4 mg/kg ofrece concentraciones séricas clínicamente eficaces y estables durante 24 horas en ratas tras cirugía de IM.