RESEARCH PAPER

Pharmacological profiles of intravenously and subcutaneously administered fentanyl in the rabbit (*Oryctolagus cuniculus*)☆

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Abstract

Objective To measure the bioavailability, maximal plasma concentration (C_{max}) and half-life ($t_{½}$) of fentanyl in rabbits after intravenous (IV) and subcutaneous (SC) administration. Secondary aims were to investigate behavioural effects and faeces production.

Study design Randomized, experimental, crossover study.

Animals A group of six male New Zealand White rabbits, Crl:KBL(NZW), aged 5−6 months and weighing 2.9−3.7 kg.

Methods Fentanyl 15 μg kg^{-1} was administered IV and SC to rabbits with a 14 day washout period. Plasma was sampled at 2 (IV), 5, 10, 15, 30, 60, 90, 120 (IV + SC), 300 (SC) and 390 (SC) minutes and analysed by liquid chromatography−mass spectrometry. Sedation was scored from 0−3, 3 being most sedated, 10 and 45 minutes after administration by an assessor blinded to treatment. Oxygen was supplemented if the haemoglobin oxygen saturation (SpO_2) was <90%. After the experiment, faeces and urine were weighed. Data are presented as mean ± standard deviation or median (range).

Results Bioavailability for fentanyl after SC administration was 47% ± 16% and 50% (27%−68%). The $t_{½}$ for fentanyl after IV and SC administration was 65 ± 11 and 275 ± 16 minutes, respectively. The C_{max} of fentanyl after SC injection was 0.55 ± 0.17 ng mL^{-1} and the time to maximal concentration (T_{max}) was 15 (15−68) minutes. Median sedation score at 10 minutes was higher ($p = 0.014$) after IV than after SC administration (3 and 1, respectively). After IV administration, but not SC administration, 5/6 rabbits became recumbent and 2/6 rabbits required oxygen. There was no statistically significant difference in faecal/urinary output between groups.

Conclusions and clinical relevance Fentanyl administered SC as the sole drug to conscious rabbits resulted in variable plasma concentrations substantially lower than after IV administration. Supplemental oxygen should be available whenever fentanyl is administered.

Keywords analgesia, bioavailability, plasma concentrations, veterinary anaesthesia.

Introduction

Rabbits are popular pets and frequently present for surgical procedures at animal hospitals. Anaesthesia and pain management in rabbits can be perceived as challenging, and perianaesthetic mortality and morbidity is high compared with that in dogs and cats (Brodbelt et al. 2008; Lee et al. 2018). It is therefore of importance to refine the anaesthetic and analgesic regimes in this species. The potent μ-receptor-agonist fentanyl is frequently used in veterinary anaesthesia for intraoperative antinociception, reduction of the minimum alveolar concentration (MAC) of isoflurane and postoperative analgesia (Barter et al. 2015; Tearney et al. 2015; Bradbrook & Clark 2018). Epidural and inhalational administration of fentanyl has been described in rabbits (Tan et al. 1996; Gusak et al. 2013). In dogs and cats, it is usually administered intravenously (IV) and has a short onset time and short duration of action (Kerr 2016). Fentanyl (20 μg kg^{-1}) administered intramuscularly (IM) in combination with midazolam and medetomidine has been reported to produce surgical anaesthesia for approximately 30 minutes in rabbits with a mean onset time of 12 minutes (Henke et al. 2005).

The subcutaneous (SC) route offers advantages over IM administration, which can cause pain and potential muscle

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damage particularly when a high volume relative to the animal's weight is administered (Diness 1985; Diehl et al. 2001). Administration of a SC bolus of fentanyl at 15 μg kg^{-1} has been explored in dogs (KuKanich 2011). Fentanyl was rapidly absorbed, and the resulting plasma concentrations were within the range associated with analgesia in humans. However, because of the limited bioavailability of some drugs administered SC, this route might not always be suitable in rabbits, as is the case for buprenorphine (Askar et al. 2020).

Fentanyl plasma concentrations have been measured in rabbits after IV, transmucosal and transdermal administration (Hess et al. 1971; Foley et al. 2001; Malkawi et al. 2008; Mirschberger et al. 2020). However, pharmacokinetic data of fentanyl after SC administration in rabbits is scarce according to database searches (PubMed, Web of Science, Scopus) covering the years 1940−2024 {search query: '[rabbit* AND fentanyl AND subcutaneous* AND (bioavailability OR pharmacokinetic*)]'} only yielding one previous publication by Ohtsuka et al. (2001). It is therefore of interest to investigate if, and for how long detectable fentanyl plasma concentrations are maintained after one dose of fentanyl administered SC in rabbits.

The main aim of this study was to measure the pharmacokinetic parameters and bioavailability of fentanyl in conscious rabbits after SC administration of 15 μg kg^{-1}. Secondary aims were to investigate the behavioural effects and effects of route of administration on faecal output. No hypotheses were formulated before the study.

Materials and methods

This study was designed as a randomized crossover pharmacokinetic study. Ethical approval was granted from Uppsala Animal Experimental Ethics Board, reference number 5.8.18-15533/2018. The ARRIVE guidelines 2.0 were followed when preparing the manuscript.

Animals

A group of six male New Zealand White rabbits, Crl:KBL(NZW), 5−6 months old, weighing 2.9−3.7 kg (mean weight 3.3 kg) and body condition score 3/5, were used. This was a convenience sample, taking the three Rs principle into account as well as the number of animals included in similar studies (Askar et al. 2020; Mirschberger et al. 2020). Before this study, the rabbits were included in a socialization study which included habituation to handling and blood sampling. More than 2 weeks elapsed between studies. According to breeder health monitoring results, the rabbit colony was free from common pathogens (Appendix A) and determined healthy upon clinical examination. Rabbits were housed singly in pens of 3 m^2 with an elevated platform, also serving as cover, autoclaved straw and wood shavings for bedding, hay and tap water in bottles *ad libitum* and a restricted amount of a pelleted diet (Lactamin K1; Lantmännen, Sweden). They were housed within smell and sight of each other.

Randomization and experiment

The six rabbits were randomly assigned an individual number from 1 to 6 in Microsoft Office Excel (version 16.0, 2016; Microsoft Corporation, WA, USA). On the first experimental day, fentanyl was administered to the rabbits in the order 1−6 whereby rabbits number 2, 4 and 6 were received the IV treatment and 1, 3 and 5 the SC treatment. On the second experimental day, the rabbits received the treatment in the reverse order (starting with rabbit 6), numbers 5, 3 and 1 were received the IV treatment and numbers 6, 4 and 2 the SC treatment. Owing to blood sampling difficulties, two rabbits were excluded on one of the experimental days and instead included on a third day. A washout period of 14 days was allowed between all 3 experimental days.

On the day of the experiments, rabbits were placed in cages (type EC2; Scanbur A/S, Denmark) marked with the assigned experimental number. Disposable bed pads were placed in the collection pan below the cage floor at the time of injection for urine and faeces collection. There was free access to pelleted diet, water, hay and straw.

An hour before catheter placement, the dorsal aspect of the pinnae were clipped, EMLA cream (lidocaine 25 mg g^{-1} + prilocaine 25 mg g^{-1}; Aspen Nordic, Denmark) was applied over the artery and veins and Elizabethan collars placed.

For the IV administration of fentanyl, rabbits were instrumented with a 22 gauge catheter (BD Venflon TM; Becton Dickinson Infusion Therapy AB, Sweden) in the auricular marginal vein. Rabbits that were scheduled to receive fentanyl SC had a catheter taped onto the pinna for the purpose of blinding. In addition, all rabbits had a 20 gauge catheter (BD Venflon TM; Becton Dickinson Infusion Therapy AB) placed in the auricular artery for blood sampling. To prevent clotting, heparin saline 100 IU mL^{-1} (Heparin LEO 5000 IE mL^{-1}; LEO Pharma AB, Sweden) was placed in the catheters between sampling time points.

For IV injection, fentanyl (Fentanyl 50 μg mL^{-1}; B. Braun Melsungen AG, Germany) 15 μg kg^{-1} was diluted with saline for injection (Natriumklorid Braun 9 mg mL^{-1}; B. Braun Melsungen AG) to a total volume of 2 mL to facilitate a standardized rate of administration. The IV injection was continuously administered from a 2 mL syringe by hand over 1 minute, followed by a saline flush of 0.5 mL. The SC administered fentanyl was not diluted and injected with a 23 gauge hypodermic needle from a 1 or 2 mL syringe depending on calculated volume. Blood samples were taken at baseline (before injection), 2, 5, 15, 30, 60, 90, 120 and 180 minutes

after IV administration. In the SC group, sampling occurred at baseline, 5, 15, 30, 60, 90, 180, 300 and 390 minutes after injection.

At each time point, 0.3 mL of blood was discarded before collecting a sample of 1.3 mL. The blood was collected in ethylenediaminetetraacetic acid tubes (EDTA KE; Sarstedt AG & Co., Germany), and the catheter flushed with saline before adding the heparin lock solution. The total volume of blood sampled during 1 experimental day was < 15 mL per rabbit, corresponding to 4.0−5.2 mL kg^{-1}. The EDTA−blood was centrifuged at 3.5 ***g*** and 5−6 °C for 15 minutes. The plasma was stored at −80 °C in cryogenic tubes (Screw cap micro tube, 1.5 mL; Sarstedt AG & Co.) until analysis was performed.

At the end of the experiment (6.5 hours post-injection), the catheters were removed, and all rabbits were administered 20 mL (~6 mL kg^{-1}) acetated Ringer's solution (Ringer acetate Fresenius Kabi; Fresenius Kabi AB, Sweden) SC to support fluid balance. At this time point, to estimate faecal and urinary output, the bed pad was weighed (PR5002 DeltaRange Mettler Toledo digital scale, Sweden) and the dry weight of the pad subtracted.

Behavioural effects

A blinded observer (AR) scored the rabbits in their cages before fentanyl administration (baseline) and 10 and 45 minutes after administration. The time points were chosen to avoid interference with blood sampling. The constructed excitation and sedation score criteria are shown in Table 1. In cases of uncertainty, the scorer was allowed to assign rabbits a decimal number between two integer scores.

Safety measures

According to the study protocol, pulse oximetry (Cardell veterinary monitor 9402; CAS Medical Systems, Inc., CT, USA), an oxygen concentrator (DeVilbiss healthcare 5 L; Drive DeVilbiss Healthcare, Germany), a face mask, a laryngeal mask (v-gel; Scandivet AB, Sweden) and naloxone were readily available in case of severe adverse reactions. It was decided that pulse oximeter readings should be obtained if animals were showing signs of marked sedation and/or respiratory depression, such as bradypnoea (respiratory rate < 30 breaths $minute^{-1}$) judged by manually counting the breaths, or dyspnoea. Supplemental oxygen was administered if pulse oximeter (SpO_2) readings were < 90%. The predetermined cut-off for naloxone (10−40 μg kg^{-1} IV to effect or 40 μg kg^{-1} SC) was apnoea for > 30 seconds or SpO_2 readings < 90% despite oxygen supplementation as judged by a board-certified veterinary anaesthesiologist. In case of apnoea, a laryngeal mask was to be placed, and ventilation assisted.

Table 1 A simple descriptive scale for subjectively scoring sedation and/or excitation constructed for the use in six New Zealand White rabbits after the administration of fentanyl 15 μg kg^{-1} intravenously (IV) or subcutaneously (SC).

Sedation score		Excitation score	
Score	Description	Score	Description
0	Sitting, attentive, chewing	0	No change in activity
1	Sitting still	1	Slight activity increase
2	Sitting, leaning head or nodding	2	Moderate excitation
3	Lying/recumbent	3	Marked excitation

'Sitting': normal sitting position for a rabbit. 'Slight', 'moderate' or 'marked' is scored at the discretion of the observer.

Plasma analysis

The plasma samples were analysed by liquid chromatography−mass spectrometry (LC-MS/MS) at a commercial laboratory (Admescope, Finland).

The bioanalytical samples were prepared for analysis by protein precipitation. A 50 μL plasma sample was mixed with 250 μL of 1% formic acid in acetonitrile containing the internal standards (50 ng mL^{-1} dextromethorphan, propranolol and phenacetin). After mixing on a tabletop shaker for 3 minutes, the samples were centrifuged at 2272 ***g*** for 20 minutes. Then 250 μL of supernatant was transferred to Waters Ostro Protein Precipitation and Phospholipid Removal plate and eluted through with Waters Positive Pressure-96 processor. Then 100 μL of eluent was transferred to an analytical plate, diluted 1:1 with ultrapure H_2O and submitted to analysis by LC-MS/MS. This method used a Waters ACQUITY UPLC + Waters Xevo TQ-S triple quadrupole MS and Waters ACQUITY BEH C18 (2.1 × 50 mm, 1.7 μm) column with precolumn filter. Standard samples were prepared by spiking 5 μL of study compound dilution to 45 μL of Sponsor blank rabbit plasma to final concentrations of 0.002−200 ng mL^{-1}. Quality control (QC) samples were prepared by spiking 5 μL of study compound dilution to 45 μL of Sponsor blank rabbit plasma to final concentrations of 0.06, 0.6, 6 and 60 ng mL^{-1} in duplicate. Standard and QC samples were otherwise prepared for analysis in the same way as the samples. The detection limit was 0.01 ng mL^{-1}, the quantitation limit 0.02 ng mL^{-1}, the standard curve range was 0.02−200 ng mL^{-1} with a coefficient of determination (R^2) of 0.998. The QC accuracy ranged from 87.8% to 105.9%.

Pharmacokinetic analysis

For each animal and route, the concentration of fentanyl was plotted *versus* time in Microsoft Office Excel. The maximal concentration of fentanyl in plasma (C_{max}), time to reach C_{max}

(t_{max}), terminal half-life ($t_{½}$) and the area under the plasma concentration curve (AUC) were calculated with a non-compartmental model using the PKSolver add-in for Microsoft Office Excel. The terminal elimination time constant (λz) was calculated using the last five time-concentrations (30–180 minutes) for all IV administrations and the last four points for SC administrations (90–390 minutes).

The AUC was calculated for both IV and SC from time 0 to the sample at 180 minutes using the linear trapezoidal method: $AUC = ½ (C_1 + C_2) (t_2 - t_1)$. The bioavailability (F) for the SC administration route for each rabbit was then calculated from the mean $AUC_{\mathbf{0\text{-}3h}}$ for IV and SC administration respectively by using the equation:

$F(\%) = 100 \times (AUC_{SC} \times dose_{IV})/(AUC_{IV} \times dose_{SC})$.

Statistical analysis

Pharmacokinetic data were summarized with descriptive statistics in Microsoft Office Excel. The nonparametric analysis module (Wilcoxon, Friedman Rank Sum test) was used to compare median sedation scores between treatments, and a paired *t*-test for comparison of faecal/urinary output predicted means (95% confidence interval) between treatments were performed in InVivoStat (ver.4; UK, Clark et al. 2012). A *p*-value < 0.05 was considered significant.

Results

Data from all rabbits were included in the fentanyl plasma concentration analysis. Blood sampling failed in one rabbit (number 5, SC administration) on the first experimental day. Data from this rabbit were included for behavioural and faecal/urinary output analysis. SC administration of fentanyl was then repeated on a third experimental day for collection of blood for pharmacokinetic data. On the second experimental day, arterial cannulation was unsuccessful in three rabbits, and instead an auricular venous catheter was placed from which blood was collected (number 4, SC administration; number 5, IV administration). This required longer sampling times and because of the limited time given the sampling interval, blood samples from one rabbit (number 1, IV administration) were excluded and instead the rabbit was enrolled in the third experimental day.

Plasma concentrations

Rabbit number 2 was excluded from the SC calculations as λz could not be calculated.

The mean and standard deviation, and median (minimum–maximum range) F% of fentanyl administered SC was 47% ± 16% and 50% (27%–68%). Pharmacokinetic data are shown in Table 2.

Mean plasma concentrations in rabbits after SC and IV administration of fentanyl are shown in Fig. 1. Individual plasma concentrations are shown in Figs 2 and 3.

Pharmacodynamic effects

All rabbits received an excitation score 0 at every time point (no excitation), hence only sedation scores are shown in Table 3. All rabbits scored 0 at baseline. Fentanyl IV produced recumbency in 5/6 rabbits. At 10 minutes, the median sedation score was significantly higher after IV (median 3) compared with SC administration (median 1), $p = 0.014$. This effect was absent at 45 minutes (median 1.0 and 1.5, respectively), $p = 0.18$. Of the six rabbits, two required oxygen supplementation after IV administration of fentanyl. Rabbit number 2 (first experimental day) lost its righting reflex shortly after IV administration and displayed miosis. It was bradypnoeic (respiratory rate 15 breaths minute^{-1}) and bradycardic with a pulse rate (PR) of 60–68 beats minute^{-1} registered from the pulse oximeter, and SpO_2 of 83%–88%. Within 1 minute of oxygen administration at 1 L minute^{-1} by mask, the SpO_2 had increased to 95%–100%. Within 10

Table 2 Pharmacokinetic parameters measured or calculated in a group of New Zealand White male rabbits administered fentanyl 15 μg kg^{-1} intravenously (IV) or subcutaneously (SC).

Parameter	SC	(*n* = 5)	IV	(*n* = 6)
	Mean ± SD	Median (range)	Mean ± SD	Median (range)
C_{max} (ng mL^{-1})	0.55 ± 0.17	0.55 (0.32–0.79)	11.55 ± 9.49	7.98 (4.38–29.9)
T_{max} (minutes)	29.1 ± 22.8	15.7 (15.3–68.0)	2.2 ± 0.2	2.2 (2.0–2.4)
$t_{½}$ (minutes)	275.1 ± 16.3	272.8 (252.3–293.4)	65.2 ± 11.4	61.1 (56.7–87.9)
V_{ss} (L kg^{-1})	-	-	5.29 ± 2.69	5.75 (1.39–8.60)
Cl (mL kg^{-1}minute^{-1})	-	-	81.0 ± 25.7	93.8 (39.6–100.6)
$AUC_{0\text{-}t}$ (minute ng mL^{-1})	118.8 ± 17.9	116.7 (95.5–141.4)	189.3 ± 91.1	137.5 (134.6–358.2)
$AUC_{0\text{-}\infty}$ (minute ng mL^{-1})	187.9 ± 25.0	178.2 (160.7–220.5)	209.0 ± 92.5	160.4 (149.1–378.9)

$AUC_{0\text{-}t}$, area under the time-concentration curve (time 0–180 minutes); $AUC_{0\text{-}\infty}$, area under the time–concentration curve (time 0–infinity); Cl, clearance; C_{max}, maximum measured fentanyl concentration; SD, standard deviation; T_{max}, time to C_{max}; $t_{½}$, elimination half-life; V_{ss}, volume of distribution at steady state.

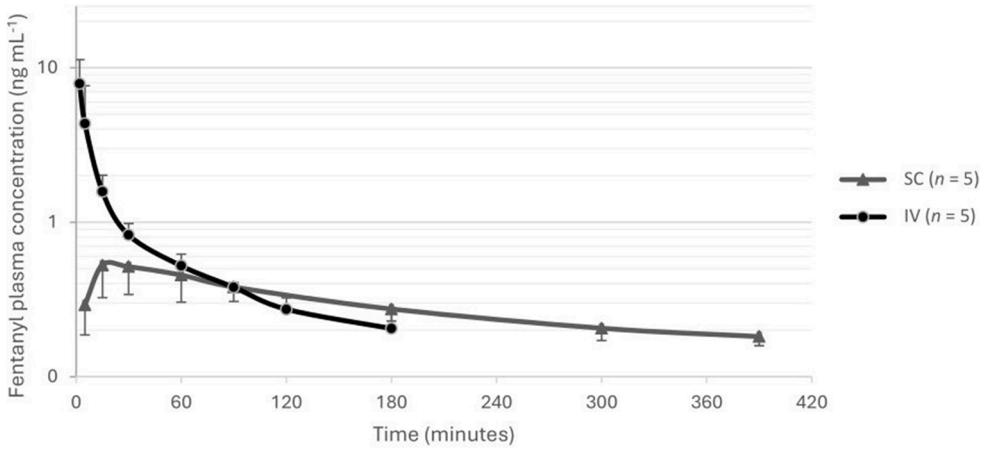

Figure 1 Mean plasma concentrations with standard deviations measured in five male New Zealand White rabbits after intravenous (black line) and subcutaneous (grey line) injections of fentanyl (15 µg kg^{-1}), respectively, at specified time points.

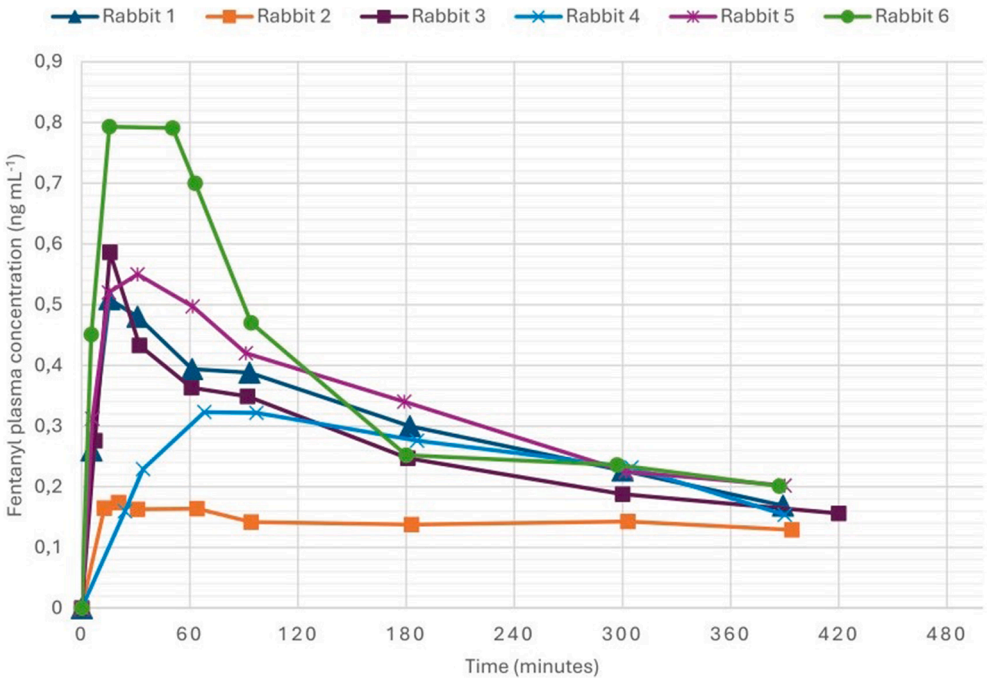

Figure 2 Individual plasma concentrations measured at specified time points in six male New Zealand White rabbits after subcutaneously administered fentanyl (15 µg kg^{-1}).

minutes, the sedation score had decreased from 3 to 2. At 45 minutes post-injection, the sedation score had decreased to 1. Rabbit number 3 (second experimental day) also lost its righting reflex at the first scoring point. The PR varied between 54 and 71 beats minute^{-1} and SpO_2 measured 55% at lowest. Immediately after oxygen administration at 1 L minute^{-1}, SpO_2 increased to 99% and the PR to180 beats minute^{-1}. The rabbit assumed a sternal recumbency within 15 minutes and the oxygen could be discontinued. At 45 minutes the sedation score was still 3, but the rabbit was aware and responsive and within 20 minutes it was fully recovered.

Faecal/urinary output data are shown in Table 3. The predicted mean difference (95% confidence interval) in faecal

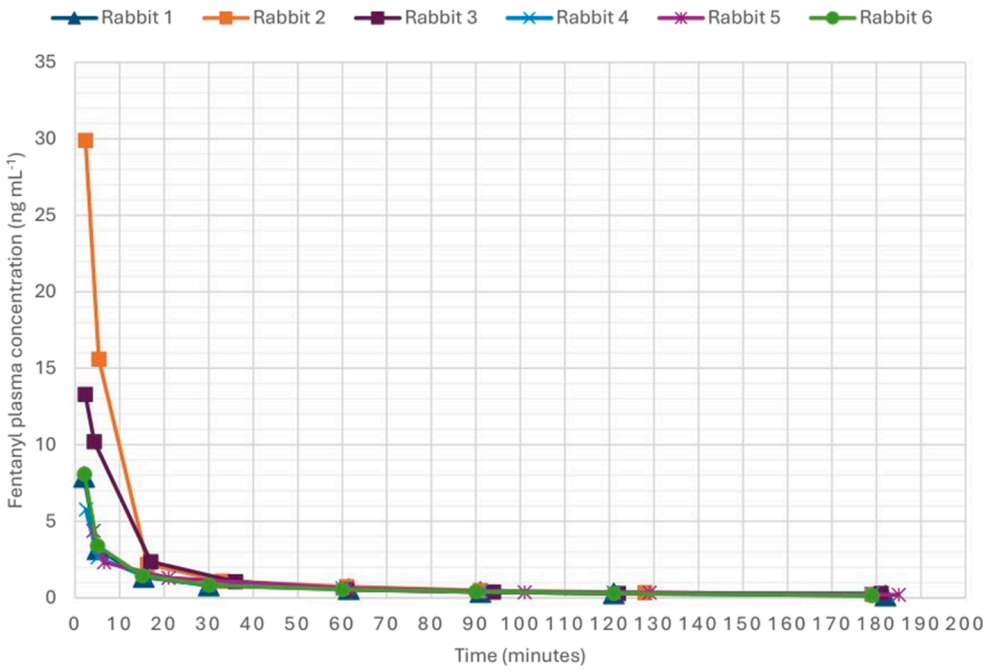

Figure 3 Individual plasma concentrations measured at specified time points in six male New Zealand White rabbits after intravenously administered fentanyl (15 μg kg^{-1}).

Table 3 Sedation scores obtained at 10 and 45 minutes and faecal/urinary output 6.5 hours after the administration of fentanyl 15 μg kg^{-1} subcutaneously (SC) or intravenously (IV) to six male New Zealand White rabbits.

Rabbit number	Injection route	Baseline score	10 minute score	Est. plasma conc. (ng mL^{-1})	45 minute score	Est. plasma conc. (ng mL^{-1})	Faecal/urinary output (g)
1	SC	0	1	0.38	2.5	0.44	17.7
	IV	0	3	–	1	–	19.5
2	SC	0	1	0.17	1	0.16	9.5
	IV	0	3	10.0	1	1.0	6.1
3	SC	0	2	0.37	0	0.40	18.8
	IV	0	3	6.50	3	0.80	5.4
4	SC	0	0	0.07	1	0.32	3.9
	IV	0	3	2.20	2	0.80	7.0
5	SC	0	0	–	0	–	30.2*
	IV	0	1	1.80	1	1.20	11.5
6	SC	0	1	0.63	1	0.76	6.6
	IV	0	3	2.40	3	0.80	6.9

Est. plasma conc., estimated plasma concentration at scoring (derived from concentration–time curves). 0: sitting, attentive, chewing; 1: sitting still; 2: sitting, leaning head or nodding; 3: lying. *Urine noticed outside of pad. Fentanyl concentrations for rabbits 1 (IV) and 5 (SC) are not stated owing to different days for scoring and blood sampling.

and urinary output between rabbits after SC and IV administration was 5.0 (−4.4 to 14.4) g, which was not statistically significant ($p = 0.228$).

All rabbits recovered well, and no long-term adverse effects were detected after the experiments.

Discussion

In this study, the bioavailability of fentanyl administered SC at the dose of 15 μg kg^{-1} was approximately 50% which is similar to that reported for buprenorphine (36%–71%) and higher than that reported for methadone (18%) in the rabbit

(Askar et al. 2020; Pujol et al. 2023). Compared with both buprenorphine and methadone, fentanyl is more lipid soluble which affects the distribution after SC injection (Valverde 2008). In rabbits, the SC route is preferable when administering a combination of drugs, owing to volume limitations with IM injection. The bioavailability after IM administered fentanyl is unknown to the best of the authors' knowledge (database search in CAB, PubMed, Scopus). However, it is probably greater because of the higher blood flow, and therefore uptake, in muscles compared with SC where fat acts as a storage site for fentanyl (Peck & Harris 2021). There was a wide variability in the fentanyl plasma concentrations achieved with the SC treatment in this study. This might be explained by regional differences in blood and lymph flow, both factors affecting SC bioavailability (Richter et al. 2012). SC injection with fentanyl at 15 μg kg^{-1} in Greyhounds resulted in a C_{max} more than six times higher than in the present study (KuKanich 2011). In humans, absorption after SC injection seems much more efficient than in rabbits (Życzkowska & Wordliczek 2009). In a study by Capper et al. (2010) involving healthy male humans administered 200 μg (2−3 μg kg^{-1}) fentanyl SC, the median C_{max} was 0.55 ng mL^{-1} and median T_{max} 15 minutes, which is identical to the findings of the present rabbit study, although the rabbits were injected with approximately six times the dose.

In this study, the half-life was noticeably longer with the SC treatment than with the IV treatment (~4.5 *versus* ~1 hour), and like that reported in a study using buprenorphine in rabbits conducted at the same institution (Askar et al. 2020). This could be a result of slow and unpredictable absorption after SC injection prolonging the elimination phase, which would also explain the high variability in T_{max} (15−68 minutes). Ideally when an opioid analgesic is included in the premedication, its analgesic effect should last throughout the procedure. Although half-life is not linearly correlated to reduction in clinical effect, it is evident that fentanyl remains in the body for several hours after SC administration. Rabbits undergoing anaesthesia for surgery may be administered other drugs, for instance an α_2-adrenoceptor agonist and inhalant anaesthesia that could affect fentanyl pharmacokinetics.

In our study, after SC injection, plasma concentrations did not reach ≥ 2.2 ng mL^{-1} reported to reduce the isoflurane MAC in this species (Barter et al. 2015). The fentanyl plasma concentrations associated with analgesia and antinociception in the rabbit are yet to be confirmed. Both the mean and median maximum plasma concentrations detected after SC administration in this study were 0.55 ng mL^{-1}. This concentration does not correspond to plasma concentrations previously reported to be associated with antinociception and analgesia in cats (> 1.07 ng mL^{-1}) or humans (0.6−3 ng mL^{-1}) (Peng & Sandler 1999; Robertson et al. 2005; Carrozzo et al. 2018). Some authors refer to 0.5 ng mL^{-1} as an analgesic plasma concentration in humans and have extrapolated this value to rabbits (Foley et al. 2001; Mirschberger et al. 2020). Lehmann et al. (1988) found even lower concentrations to be effective in some human patients but also found large inter-individual variability in effective concentrations. This is probably true for animals as well since pain is defined as an individual experience (IASP 2021). Also, different types of pain often require different levels of analgesia (Monteiro et al. 2023). Therefore, analgesics should ideally be administered after pain assessment or pre-emptively before surgery, and the pain scores reassessed. However, rabbits normally hide signs of pain which makes assessment and individually adjusted dosing challenging (Weaver et al. 2010; Benato et al. 2019), even though pain scales can offer support.

We chose the dose of 15 μg kg^{-1} because it is similar to previously reported doses (Henke et al. 2005; Baumgartner et al. 2010), and the same dose has been investigated in dogs (KuKanich 2011). However, this dose is too high to be recommended for IV bolus injection in a clinical setting because two of six rabbits were immediately heavily sedated with bradycardia and hypoxaemia as judged by pulse oximeter readings. Bradycardia and respiratory depression are two well-known side effects of fentanyl (Kukanich & Clark 2012). In a study performed in Greyhounds, the same IV dose was reduced to 10 μg kg^{-1} because the dogs were heavily sedated with dysphoria when fentanyl was combined with midazolam (KuKanich & Hubin 2010). Other authors have used similar doses IV but in ventilated and/or anaesthetized rabbits (Rigg et al. 1981; Malkawi et al. 2008). Apnoea was reported in rabbits after administration of 20 μg fentanyl kg^{-1} IM in combination with midazolam and medetomidine (Henke et al. 2005). Foley et al. (2001) reported respiratory depression in two rabbits after application of a fentanyl patch, and these rabbits had similar or lower C_{max} values compared with rabbits administered IV fentanyl in our study. To determine the SC bioavailability, the same dose was administered IV. In clinical practice, it would be more reasonable to use a lower IV dose, preferably tailored to the desired analgesic effect, thereby avoiding adverse effects. It is evident from Table 3 that rabbits 2 and 3, which both required oxygen, had the highest initial plasma concentrations of fentanyl. If fentanyl were included in an anaesthetic plan, supplemental oxygen, means of securing the airway with access to mechanical ventilation as well as reversal agents should be available. In contrast to IV administration, SC sedation scores were lower probably because of lower plasma concentrations.

Rabbit number 2 was characterized as an outlier and was excluded from SC pharmacokinetic data because of inability to establish λz. The detected plasma concentrations after SC injection in this rabbit showed a seemingly flat concentration−time curve. The relatively small changes in

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concentrations could potentially be attributed to more SC fat, but this rabbit was of a similar size to the others, although it had marginally higher body weight (3.72 kg). Other hypothetical explanations are undetected inadvertent cutaneous injection, failure to inject the full volume when piercing the skin or alternatively a calculation error. The high concentration in rabbit number 2 at 2 minutes after IV administration could potentially be owing to contamination of the sample (for instance, sampling through the wrong catheter) or failure of accurate dilution. However, a C_{max} of 730 ng mL^{-1} was reported after a 50 μg fentanyl IV bolus in rabbits, which is similar to the dose we used, bearing in mind the differences in analytical assay methodology (Malkawi et al. 2008). The variations in early plasma concentrations after IV injection seen in our study and in other studies, are proposedly as a result of unequal central compartment mixing and pulmonary uptake (Hess et al. 1971; Rigg et al. 1981). The short duration of action of fentanyl in humans is mainly owing to distribution. However, the rapid decline in fentanyl plasma concentration after an IV bolus in rabbits probably also results from more rapid metabolism compared with humans, as seen by the steeper slope of the terminal elimination curve (Hess et al. 1972). A similar rapid decline could also be seen in conscious dogs after an IV bolus of 10 μg kg^{-1} with an elimination half-life ($t_{½}\beta$) of 45.7 minutes (Sano et al. 2006).

Owing to failure of arterial blood sampling, venous blood was sampled from two rabbits. Including assay results from both venous and arterial blood in this study is considered a limitation as differences in concentrations can be expected (Huang & Isoherranen 2020).

There was a tendency towards lower faecal/urinary output in the IV group compared with the SC group albeit not statistically significant. There is a possibility that this variable could be more rabbit-dependent than intervention-dependent. This study was however likely underpowered for faecal output comparison.

Other study limitations include the sedation scoring, which was also likely underpowered, no control group was included and a nonvalidated scale was used, hence neither repeatability nor reliability can be guaranteed. Assessment of behavioural effects was however a secondary aim of this study. In terms of generalizability, this experimental study included young, healthy, conscious male laboratory New Zealand White rabbits. It might be inappropriate to directly extrapolate the data to clinical patients of other breeds as both age and comorbidities might affect the pharmacokinetics and pharmacodynamics of fentanyl. Furthermore, direct application to inbred or genetically modified laboratory rabbits, female or anaesthetized rabbits should be done with caution.

Conclusion

Administering 15 μg kg^{-1} fentanyl SC as a sole drug to rabbits is associated with limited bioavailability and unpredictable plasma concentrations unlikely to be MAC-sparing. Additional analgesia for surgery could therefore be needed depending on the severity and character of the expected nociceptive stimulation. Supplemental oxygen should be available whenever fentanyl is included in the anaesthetic protocol.

Conflict of interest statement

The authors declare no conflict of interest.

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Authors' contributions

VB: study design, conducting experiment, data interpretation, preparation of manuscript and giving final acceptance to the manuscript. LO: study design, conducting experiment, data interpretation, pharmacokinetic analysis, preparation of manuscript and giving final acceptance to the manuscript. AR: sedation scoring, reviewing the manuscript and giving final acceptance to the manuscript. JB, ME and EG: conducting experiment, reviewing the manuscript and giving final acceptance to the manuscript. PH: study design, conducting experiment, data interpretation, statistical analysis, preparation of manuscript and giving final acceptance to the manuscript.

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