The Use of Minipigs for Testing the Local Intranasal Toxicity of Fentanyl

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Summary

The local intranasal toxicity of a nasal formulation of the opioid analgesic fentanyl was investigated in this study. Minipigs were used as the experimental model. Fentanyl was administered using the formulation and the device intended for human use. Doses of 400 µg fentanyl were administered 5 times daily to the minipigs for 4 weeks. In addition, the spreading pattern of the formulation in the minipig nasal cavity was investigated by applying a formulation containing methylene blue at necropsy.

Results: The methylene blue spread to the middle parts of the endoturbinates. The No-Observed-Adverse-Effect-Level (NOAEL) of intranasally administered fentanyl in minipigs was documented to be above 5 x 400 µg/day. After necropsy, no relevant treatment related macroscopic or microscopic findings were observed, but minimal focal deciliation/degradation of the respiratory epithelium was seen in one animal. In conclusion, intranasal administration of 400 µg fentanyl 5 times daily for a period of 4 weeks did not cause any treatment related changes in the nasal cavity of the minipig.

Introduction

Local intranasal toxicity caused by nasal formulations has been evaluated in a number of different animal species (*Gizurarson, 1993; Gizurarson, 1996; Hjortkjaer et al., 1999; Ugwoke et al., 2001*). The objective of this study was to asses the local intranasal toxicity of fentanyl after intranasal administration.

The lipophilic analgesic opioid fentanyl has been shown to have a relatively high nasal bioavailability in humans (*Paech et al., 2003; Striebel et al., 1993*) and therefore the bioavailability is also expected to be high after intranasal administration in an animal model.

Due to the expected high nasal bioavailability, the intention of administering high doses of fentanyl frequently over a 4-week period using the formulation and the nasal device intended for human use, it is required to use an animal model of a certain size.

Furthermore, the nasal cavity of the pig/minipig has anatomical similarity with the human nasal cavity, and the docile nature of the minipig makes the frequent and repeated dosing possible and easy. Therefore, minipigs with a body weight above 25 kg were used as the model species in this study.

Initially, a pilot study was conducted to consider the No-Observed-Adverse-Effect-Level (NOAEL) of intranasal fentanyl in minipigs in the range of 100-400 µg, which was within the dose range intended for clinical use in humans. Furthermore, the pilot study was initiated to investigate the distribution of the test formulation in the nasal cavity.

After assessment of the NOAEL, a main study with a dosing of 400 µg fentanyl five times daily with approximately 4 hours interval for a period of 4 weeks, followed by a 2 weeks recovery period was conducted. The dosing regime in the main study

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Published in the Scandinavian Journal of Laboratory Animal Science - an international journal of laboratory animal science

was chosen to resemble the clinical situation when nasal fentanyl is used to treat breakthrough pain in cancer patients.

Materials and methods

Materials

The studies were conducted at Scantox A/S, DK-4623 Lille Skensved, Denmark.

Animals

Pilot study: 4 female Göttingen SPF minipigs from Ellegaard Göttingen Minipigs ApS, Dalmose, Denmark were used as test animals.

At the start of the acclimatisation period, the animals were 13.5 to 14.5 months old and had a body weight in the range of 27.0 to 27.1 kg.

Main study: 12 female Göttingen SPF minipigs from Ellegaard Göttingen Minipigs ApS, Dalmose, Denmark were used as test animals.

At the start of the acclimatisation period, the animals were 8 to 13 months old and had a body weight in the range of 24.8 to 30.5 kg.

Housing

Both studies were performed in an animal room provided with filtered air at a temperature of 21±3˚C, a relative humidity of 55±15% and a ventilation system giving 10 air changes per hour. The room was illuminated to give a cycle of 12 hours light and 12 hours darkness.

The animals were housed individually in floor pens (1.2 m^2) with sawdust ("Lignocel 3-4" from J. Rettenmaier Söhne GmbH & Co, D-73494 Rosenberg, Germany) as bedding.

Diet

In both studies, 400 g per animal/meal SDS minipig diet (SMP MOD from Special Diets Services, Witham, Essex, CM8 3 AD, U.K.), was offered twice daily. A supply of autoclaved hay was given daily. Twice daily the animals were offered domestic quality drinking water.

Chemicals

The following chemicals were used: fentanyl citrate (Mallinckrodt Chemical Limited, Derbyshire, UK), sodium dihydrogen phosphate dihydrate (Merck KGaA, Darmstadt, Germany), disodium phosphate dihydrate (Chemische Fabrik Budenheim, Budenheim, Germany), methylene blue (Merck KGaA, Darmstadt, Germany), Zoletil 50 vet., (Virbac, France), Rompun Vet. (Bayer, Germany), Ketaminol Vet. (Veterinaria AG, Switzerland) and Methadon (Nycomed Danmark, Denmark)

Delivery device and dosing procedure

The device consisted of a Valois CB18 actuator, a Valois 100 µl VP7D pump (Valois Division Pharmacie, Le Vaudreuil, France) and a brown (45x24x1.2 mm) vial (Münnerstädter Glaswarenfabrik GmbH, Münnerstadt, Germany).

Before the first use, the device was primed. The correct delivery of the formulation was obtained by bending the head of the animal slightly, inserting the device approximately 1.5 cm into the nostril, ensuring that the device was not angled more than 45 degrees from the upright position, and activating the spray.

The weight of each spray device before and after dosing was documented to verify the delivery of dose from the device. In cases where the administered dosing volume was below 50 µl, the animals were re-dosed.

Treatment

Pilot study: The animals were treated with aqueous fentanyl formulations containing fentanyl in a concentration range from 100 to 400 µg/100µl. The formulations were buffered with sodium dihydrogen phosphate dihydrate and disodium phosphate dihydrate to a pH between 6.0 and 7.0. At necropsy, all animals were treated intranasally in the left nostril with a methylene blue solution (0.2 % w/v) in placebo (see the composition below) to investigate the distribution in the nasal cavity.

The dose levels, dosing frequency and animal numbers are described in Table 1:

Table 1. Dosing regime in the pilot study.

Animal Nos 1 and 2.			
Day	Dose (µg Fentanyl/treatment)	Nos of daily dosings	Animal Nos
1	100	5	1-2
2	150	5	1-2
3	200	5	1-2
4	300	5	1-2
5	400	5	1-2

Animal Nos 3 and 4.			
Day	Dose (µg Fentanyl/treatment)	Nos of daily dosings	Animal Nos
1	400	5	3-4

If any signs of toxicity occurred in the pilot study, the maximum tolerated dose was considered reached and no further increase of dosage was performed. Animal Nos 1 and 2 were treated with the increasing doses of fentanyl. Once the maximum tolerable dose or the maximum planned dose was reached, animal Nos 3 and 4 (fentanyl naive animals) were administered this dosage 5 times on a single day with intervals of 4 hours.

Main study: The animals were treated with an aqueous fentanyl formulation containing 400 µg fentanyl per 100 µl buffered with sodium dihydrogen phosphate dihydrate and disodium phosphate dihydrate. The placebo formulation consisted of water buffered with the same excipients.

The dose levels, dosing frequency and animal numbers are described in Table 2:

All animals were treated for 28 days. Animals 1-4 and 7-10 were treated until the day before necropsy, whereas animals Nos 5-6 and 11-12 were allowed a recovery period of 14 days.
On each day of treatment, the animals were dosed 5 times with an interval of approximately 4 hours.
Treatment was performed in the period between 07:00 and 23:00 to simulate clinical practice.

Clinical observations
In both studies all visible signs of ill health and behavioural changes were recorded daily. All animals were weighed on arrival, on the first day of

Table 2. Dosing regime in the main study.

Treatment		Dose (µg fentanyl/treatment)	Dose volume (µl/treatment)	Nos of daily dosing	Animal Nos	
Right nostril	Left nostril				Main study	Recovery
Fentanyl	Untreated	400	100	5	1-6	5-6
Fentanyl	Placebo	400	100	5	7-12	11-12

treatment and weekly hereafter. The consumption of food was estimated daily for each animal by weighing the unconsumed diet.

Terminal observations

On the day of necropsy the animals were weighed, examined externally, anaesthetised with an intramuscular injection of Zoletil 50 vet., Rompun Vet., Ketaminol Vet. and Methadon and sacrificed by exsanguination during anaesthesia. The animals were sacrificed and necropsied in a non-randomised sequence of one animal/group.

Necropsy

In both studies, a macroscopic examination was performed on the visible part of the intranasal epithelium after sampling of the nasal cavity as a block. Any macroscopic change was recorded with the details of the location, colour, shape and size. For the pilot study, the distribution of the methylene blue solution in the left side of the nasal cavity was furthermore described.

Tissue sampling

In the main study, tissue for histopathological examination was sampled at 3 different levels (cranial, intermediate and caudal) in the right nostril (fentanyl treated) and in the left nostril (placebo treated or untreated control). For the pilot study, no tissues were sampled for histopathological examination.

Tissue processing and microscopic examination

All tissue sampled were fixed in a phosphate-buffered neutral 4% formaldehyde solution and decalcified. The samples were trimmed and representative specimens were taken for histopathological processing. The specimens were embedded in paraffin wax, cut at a nominal thickness of approximately 5 µm, stained with haematoxylin and eosin, and examined under the light microscope.

The histological alterations were graded on a 5 grade system:

Grade 1: Minimal/very few/very small
Grade 2: Slight/Few/Small
Grade 3: Moderate/moderate number/moderate size
Grade 4: Marked/Many/Large
Grade 5: Massive/Extensive number/Extensive size
Present: Finding present – not scored

Results

Pilot study

No treatment related clinical signs were seen in any of the animals during the study; changes in body weights were insignificant and no macroscopic changes of the nasal cavity were seen.

The distribution of the methylene blue extended to the rostral part of the endoturbinates of the nasal cavity in two of the animals, whereas a wider distribution up to the middle part of the endoturbinates was seen in the other two animals. The planned maximum fentanyl dose of 400 µg/treatment, administered five times daily at four hours intervals was reached and the No-Observed-Adverse-Effect-Level (NOAEL) was considered to be above 5 x 400 µg/treatment/day.

Main study

No treatment related clinical signs were observed during the study and the changes in body weight were insignificant. Four out of the 12 animals had short periods where the food consumption was slightly reduced. At necropsy no macroscopic changes were observed in the nasal cavity. No treatment related microscopic findings were observed. Minimal focal deciliation/degradation of the respiratory epithelium of the septal wall and the ventral nasal concha was seen in the fentanyl treated nostril of animal No 4 (Grade 1).

Slight focal deciliation/degeneration of the respiratory epithelium of the ventral nasal concha was seen in the vehicle treated nostril of animal No 7 of group 2 (Grade 2). These changes were considered to be incidental, as the two lesions were comparable but higher graded in the placebo treated nostril (Animal No 7) than in the fentanyl treated nostril (Animal No 4). No other changes were observed.

Discussion and conclusion

Rabbits, guinea-pigs and dogs have earlier been used as test animals for local nasal toxicity (*Gizurarson, 1993; Gizurarson et al., 1996; Hjortkjaer et al., 1999; Ugwoke et al., 2001*). The minipig was chosen as the test species due to the possibility to use the device, dose and formulation intended for humans. Furthermore, in this specific case where the high nasal bioavailability of fentanyl (*Paech et al., 2003; Striebel et al., 1993*) combined with the systemic toxicity of the compound could cause problems if using a smaller test species, the use of minipigs of this size as an animal model is ideal.

The pilot study revealed that it was practically possible to use the device in the minipig nostril. The spreading of the formulation as revealed by dosing methylene blue after necropsy was up to the middle part of the endoturbinates. It should be emphasized, that the spreading pattern seen was without the effect of any aspiration, as the methylene blue was administrated after the sacrifice of the animals. The spreading pattern of a formulation in the nasal cavity of model animal have not been reported earlier, even though spreading is an important factor when evaluating the local nasal toxicity.

In conclusion, the main study showed that intranasal administration of 400 µg fentanyl 5 times daily over a period of 4 weeks in the minipig caused no treatment related macroscopic or microscopic changes in the nasal cavity. No signs of systemic toxicity were observed at this dosage. It is furthermore concluded that the minipig constitutes a suitable animal model for these types of studies.

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