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Research article

Comparison of three preclinical models for nausea and vomiting assessment☆

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ABSTRACT

Introduction: Nausea is a subjective sensation often preceding emesis in humans. Drug-induced nausea remains difficult to predict in preclinical tests. The aim of this study was to compare the effects of emetic agents in rats (pica behavior), ferrets (acute and delayed phases of emesis) or dogs (emesis and cardiovascular endpoints).
Methods: Rats and ferrets were administered cisplatin (± aprepitant/ondansetron or aprepitant) or apomorphine (± domperidone). Telemetered dogs were administered apomorphine (± domperidone). Food and kaolin intake was measured in rats whereas the number of emetic events was counted in ferrets and dogs. Cardiovascular changes were also monitored in dogs.
Results: In rats, cisplatin (6 mg/kg, i.p.) increased kaolin intake (+2257%, $p < 0.001$). The cisplatin effects were not reversed by the combination of aprepitant/ondansetron (2 mg/kg, p.o./2 mg/kg, i.p.) or by aprepitant (30 mg/kg, p.o.). Apomorphine (10 mg/kg, i.p.) did not induce pica behavior. In ferrets, cisplatin (8 mg/kg, i.p.) induced acute and delayed emesis (371.8 ± 47.8 emetic events over 72 h) which was antagonized by aprepitant (1 mg/kg, p.o.). Apomorphine (0.25 mg/kg, s.c.) induced acute emesis (38.8 ± 8.7 emetic events over 2 h) which was abolished by domperidone (0.1 mg/kg, s.c.). In dogs, apomorphine (100 μg/kg, s.c.) induced emesis and tachycardia which were decreased by domperidone (0.2 mg/kg, i.v.).
Conclusions: The assessment of emesis in the ferret or in the dog displays a strong predictive value. In contrast, assessing nausea remains challenging in all animal species and the use of pica behavior remains questionable in the context of antiemetic drug development.

1. Introduction

Nausea, which is an unpleasant sensation often associated with the urge to vomit and vomiting (the forceful oral expulsion of upper gastrointestinal tract contents) are commonly encountered in clinical practice. Therefore, there is a continuing requirement to develop novel anti-emetic agents in diverse clinical situations. In addition, drug-induced nausea is a widely reported adverse effect of new chemical entities (Percie du Sert, Holmes, Wallis, & Andrews, 2012). There is thus a need for preclinical models able to detect nausea/vomiting. However, the selection of an appropriate drug development strategy in this area is intensely debated due to limitations of existing preclinical tests (Andrews & Sanger, 2014).

The rat, one of the most common laboratory animal species, cannot be used for a direct assessment of emesis because the majority of rodent species are unable to vomit (Horn et al., 2013). Nevertheless, alternative rodent models are available for nausea assessment, such as conditioned taste/flavor aversion, conditioned gaping or pica behavior (Garcia, Hankins, & Rusiniak, 1974; Parker, 2014; Yamamoto, Nakai, Nohara, & Yamatodani, 2007). In particular, the pica behavior, characterized by eating of non-nutritive material can be induced in the rat by stimuli that commonly cause emesis in species possessing an emetic reflex (Yamamoto et al., 2007).

Ferrets, dogs or house musk shrews which have a functional emetic reflex are currently used in preclinical pharmaceutical toxicology studies for the investigation of emesis. The ferret is considered as the gold standard for the pharmacological evaluation of emetic or anti-emetic drugs (Florczyk, Schurig, & Bradner, 1982) because of the high sensitivity of its vomiting reflex (Andrews & Horn, 2006). Episodes of emesis are characterized by rhythmic abdominal contractions that are either associated with the oral expulsion of solid or liquid material from the gastrointestinal tract or occur in absence of vomiting (i.e. retching movements) (Rudd & Naylor, 1996).

The aim of this study was to compare the effects of emetic and anti-emetic substances in three species (rat, ferret and dog) using three different testing procedures. We evaluated 1) the effects of cisplatin (with or without aprepitant) on the kaolin consumption (pica behavior) in the rat and on the acute and delayed phases of emesis in the ferret and 2) the effects of apomorphine (with or without domperidone) on kaolin consumption in the rat, early emesis in the ferret and emesis and

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cardiovascular endpoints in the dog. The sensitivity of the three animal models is discussed with respect to their validity for the prediction of nausea and/or vomiting during drug development. A characteristic of our study is that it allows a face-to-face comparison of the data obtained in different pre-clinical models whereas the data available in the literature generally come from separate studies, thereby complicating the comparison of the predictive validity of the different models.

2. Materials and methods

2.1. Animals

During the period of habituation to the environmental conditions of our laboratory, male Wistar rats (Charles River, France) weighing 214–284 g (on the third day of the habituation period) were individually housed with free access to food (code 113 – Safe, France) and tap water. Male *Mustela putorius furo* ferrets (Marshall Europe, France) weighing 1.280–1.940 kg (on the day of the experiment) were group-housed with free access to food (SDS 807000, France) and tap water. From surgery to the end of the experiments, the ferrets were housed in individual cages. The animal rooms were maintained under artificial lighting between 7:00 and 19:00 at a controlled ambient temperature of 21 ± 3 °C (rat) or 18 ± 3 °C (ferret).

Before the start of the experiments, male Beagle dogs (CEDS, France) weighing 11.3 and 18.6 kg (on the day of the experiment) were housed in groups in a kennel, under natural lighting. The animal room was maintained in a controlled ambient temperature of 18 ± 3 °C. The day before surgery, each dog was housed singly in an individual box.

The experiments were performed in accordance with French legislation concerning the protection of laboratory animals and in accordance with a currently valid license for experiments on vertebrate animals, issued by the French Ministry for Agriculture and Fisheries.

2.2. Surgical techniques

2.2.1. Ferret

Ferrets (only those included in the second experiment evaluating the acute and delayed phases of emesis) were anesthetized with isoflurane and given 7.5 mg/kg s.c. carprofen. Following a midline incision in the abdomen, a TL11M2-C50PXT implantable telemetry device (Data Sciences International, France) was introduced into the peritoneal cavity, as previously described (Goineau, Rompion, Guillaume, Barrais, & Castagné, 2013). In brief, the free end of the pressure catheter was positioned into the abdominal cavity, and the tab located on the device body anchored to the inner abdominal wall. The abdominal and skin incisions were then closed. The animals were given 100 mg/kg i.m. amoxicillin and returned to their individual cages. Forty eight hours later, they were given 100 mg/kg s.c. amoxicillin. Fourteen ferrets were instrumented. Animals were allowed to recover for at least 7 days.

2.2.2. Dog

The day prior to surgery, a trans-dermic patch of fentanyl (Durogesic®) was applied to the animals. Six dogs were anesthetized (sodium pentobarbital 30 mg/kg i.v.). Following an incision in the flank and in the inguineal area, a DSI TL11M2-D70-PCT (Data Sciences International, France) implantable telemetric device was introduced into a pouch under the skin and the catheter of the device was then inserted facing upstream into a femoral artery. The biopotential positive and negative leads were coiled into a loop, after having been passed subcutaneously, and anchored to surrounding tissue in lead II configuration. The tab located on the device body was sutured to the inner abdominal wall. The skin incisions were then closed. Animals were given anti-inflammatory (carprofen) and antibiotic (amoxicillin) treatments, before, during or after surgery. The fentanyl patch was removed at least two days after surgery. Animals were allowed to recover for at least 2 weeks.

2.3. Treatment and experimental design

2.3.1. Pica behavior test in the rat

During a 3-day habituation period, rats were individually housed in new polycarbonate cages (48 × 37.5 × 21 cm) and acclimated to new environmental conditions (no litter, no enrichment). Each cage had a wire-mesh floor to permit collection of spilled food and kaolin. The rats had free access to water, kaolin pellets as well as normal food pellets in separate stainless steel containers. The food hoppers (12.6 × 11.6 × 13 cm) were placed at the level of the cage floor in order to facilitate the access to food or kaolin. Kaolin pellets were provided ready-to-use (Research Diets ref. K50001). From Day-1 and for 4 consecutive days, the kaolin and food pellets intakes were measured over 24-hour periods. The kaolin remaining in the container was weighed. In addition, the kaolin spilled on the floor of the cage was separated from waste, dried and weighed. The amount of food intake was measured in the same manner.

As already described (Tatsushima et al., 2011), only rats which ate <1 g of kaolin per day on the last 3-day adaptation were used for substance evaluation.

The following treatments were evaluated:

- cisplatin (2, 4 and 6 mg/kg, i.p.).
- aprepitant (10 or 30 mg/kg, p.o.) administered 60 min before and then 24 and 48 h after cisplatin (6 mg/kg, i.p.).
- ondansetron (2 mg/kg, i.p.) administered 10 min before cisplatin (6 mg/kg, i.p.) in combination with aprepitant (2 mg/kg, p.o.) administered 60 min before and then 24 and 48 h after cisplatin.
- domperidone (2 mg/kg, i.p.) administered 10 min before apomorphine (10 mg/kg, i.p.) for 3 consecutive days.

Cisplatin and apomorphine were administered 1 h before the dark cycle.

The doses were selected from the literature (Takeda, Hasegawa, Morita, & Matsunaga, 1993; De Jonghe & Horn, 2008; Tatsushima et al., 2011; Yamamoto et al., 2014). The substances were administered by the intraperitoneal route of administration, except for aprepitant which was administered by oral gavage (5 ml/kg).

2.3.2. Emesis in the ferret

In a first experiment only focused on the acute phase of emesis, ferrets (non-implanted with telemetry devices) were placed in individual cages without access to food. Sixty minutes later, they were treated with domperidone (0.1 mg/kg, s.c.) and with apomorphine (0.25 mg/kg, s.c.) 30 min later. Animals were then immediately observed over a 2-hour period for:

- Latency to first retching or vomiting (h:min:s),
- Retching (number of retches),
- Vomiting (number of vomits),
- Number of emetic periods.

Retching was defined as a rhythmic respiratory movement against a closed glottis, while vomiting was defined as a forced expulsion of upper gastrointestinal contents.

In a second experiment evaluating the acute and delayed phases of emesis, the individual cages of telemetered ferrets were placed near a telemetry receiver (Data Sciences International, France). After recording of the pressure signals for 60 min (IOX version v2.8.2.4., Emka Technologies, France), aprepitant (0.03 or 1 mg/kg, p.o.) was administered 2 h before and then 24 and 48 h after cisplatin (8 mg/kg, i.p.). Pressure signals were recorded up to 72 h post-cisplatin dosing in unrestrained animals. The following parameters were recorded:

- Latency to first retching or vomiting event,
- Number of emetic events (retches and vomits per epoch of 6 h),