Journal of Pharmacological and Toxicological Methods 68 (2013) 160–165

Contents lists available at SciVerse ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

# Using telemetry to automate the detection of emesis in the ferret: New vistas for delayed emesis assessment

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ARTICLE INFO

*Article history:*
Received 19 December 2012
Accepted 31 March 2013

*Keywords:*
Chemotherapy
Cisplatin
Automated detection
Emesis
Ferret
Telemetry

ABSTRACT

**Introduction**: Nausea and vomiting are common side effects of cancer chemotherapy. The ferret is a highly appropriate animal species to evaluate both early and delayed emetic events occurring hours and days after administration, respectively. If early emesis can be easily investigated in ferrets by direct observation, alternative methods are required to quantify delayed emesis. This study was designed to validate a new method of automated detection of abdominal pressure changes related to retches or vomits induced by a cytotoxic substance in the ferret. **Methods**: Five ferrets implanted with telemetry devices (Data Sciences International) were challenged with cisplatin (8 mg/kg, i.p.) and abdominal pressure was recorded in unrestrained animals for 72 h. The pressure signals were analyzed both manually and automatically using an adapted version of ecgAUTO software (Emka Technologies). Over the first 3 h, the emetic response was also quantified via direct observation of the animals. The data produced by the 3 methods of detection were compared using a Spearman's rank correlation coefficient. **Results**: Visual, manual and automated detections of early emetic events over the first 3-hour recording period were well correlated when compared per 30-, 15- or 5-minute epoch: correlation coefficients ranging from 0.8640 to 0.9289, $p < 0.0001$ for all comparisons. Manual and automated detections of early and delayed emetic events over the 72-hour recording period were also well correlated when compared per 3-hour epoch: correlation coefficient = 0.9190, $p < 0.0001$. **Discussion**: These findings demonstrate that automated detection of abdominal pressure changes with adapted software is a reliable method for measuring emetic events in the ferret. The results obtained open major possibilities for the rapid, comprehensive and objective analysis of delayed emesis. They should thereby facilitate the development of novel chemotherapeutic agents and anti-emetic therapies.



## 1. Introduction

Chemotherapy-induced nausea and vomiting is a major side-effect in the treatment of patients with cancer. Cisplatin is one of the most commonly used cytotoxic drugs in chemotherapy even though it induces severe adverse events including immediate emetic response from the first day of treatment (acute emesis) and a continuing response up to several days later (delayed emesis) (Ballatori et al., 2007; Rojas & Slusher, 2012; Schmoll et al., 2006; Tsukuda et al., 2009). Other agents such as cyclophosphamide or carboplatin can also cause delayed emesis (Roila, Donati, Tamberi, & Margutti, 2002). Despite progress in the management of chemotherapy-induced emesis with 5-hydroxytryptamine$_3$ (5-HT$_3$) antagonists, neurokinin-1 antagonists and corticosteroids (Hesketh, 2008), the control of delayed emesis remains a major challenge.

The ferret is considered as a valid model 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 (i.e. vomiting), or not (i.e. retching movements) (Rudd & Naylor, 1996). Episodes of emesis in the ferret (number of retches or vomits) are easily measured over 3–4 h by a trained observer. Direct observation is therefore sufficient and appropriate for quantifying early emesis. On the other hand, alternative methods are required to quantify delayed emesis which can occur several hours or days after administration. Some authors have recorded on video animal behavior over many hours and analyzed off-line the video at the completion of the experiment (Rudd & Naylor, 1996; Watanabe et al., 2008). This method is however very time-consuming. In addition, depending on the position of the animal in the observation cage, the visualization and therefore the evaluation of the number of emetic events can be challenging.

Another approach is to implant ferrets with telemetry devices linked to an electrode placed into the abdominal muscles or to a pressure catheter placed into the abdominal cavity. Vomits and retches can be differentiated by signature signals. This method has already been used in the ferret and the piglet by other authors who manually

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http://dx.doi.org/10.1016/j.vascn.2013.03.010

analyzed the data at the completion of the experiments (Milano, Blower, Romain, & Grélot, 1995; Percie du Sert, Chu, Wai, Rudd, & Andrews, 2009). In particular, the team of Percie du Sert investigated the acute gastric myoelectric activity and intra-abdominal pressure changes induced by emetic substances (apomorphine and cisplatin) in the ferret (Percie du Sert et al., 2009). In this study, the telemetered data were analyzed "semi-automatically" by visual analysis of the traces for each ferret. However, continuous recordings over many hours generate a huge volume of data and visual/manual analysis of traces is also very time-consuming. There remains therefore a pressing need for a fully automated method of signal analysis to optimize the ferret model and make it more suitable for pharmacological research on delayed emesis.

The following paper describes an adapted version of ecgAUTO software developed in collaboration with Emka Technologies, and presents data to validate the automated detection of abdominal pressure changes related to retches or vomits in telemetered ferrets challenged with cisplatin.

## 2. Materials and methods

### 2.1. Animals

Male *Mustela putorius furo* ferrets (Marshall Europe, France) weighing 1.1–1.3 kg when challenged with cisplatin were used.

Animals were delivered to the laboratory at least 5 days before the experiments during which time they were acclimatized to laboratory conditions. On arrival, animals were housed in one group in a room containing wood litter (Litalabo — SPPS, France) 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 room was maintained under artificial lighting between 7:00 and 19:00 at a controlled ambient temperature of 18 ± 3 °C and relative humidity between 20 and 80%.

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

Five ferrets 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. 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. Animals were allowed to recover for at least 10 days.

### 2.3. Experimental design

Ferrets were placed in their individual cages near a telemetry receiver (Data Sciences International, France) and the baseline pressure signals were recorded for approximately 60 min, with a sampling frequency of 500 Hz, using specialized software (IOX version v2.8.2.4., Emka Technologies, France).

Ferrets were then challenged with cisplatin at 8 mg/kg, i.p. and pressure signals were continuously recorded for 72 h in unrestrained animals. The most common dose of cisplatin used in ferrets to induce acute emesis is 10 mg/kg (Percie du Sert, Rudd, Apfel, & Andrews, 2011). However, because of cisplatin toxicity (Cepeda et al., 2007), lower doses were used for the evaluation of early and delayed emesis. Although the majority of the authors use a dose of cisplatin at 5 mg/kg for the evaluation of early and delayed phase (Chu et al., 2010; Rudd & Naylor, 1996; Tattersall et al., 2000), we decided to choose a dose slightly higher (8 mg/kg). Indeed, in order to compare in a reliable manner the different methods of analysis of abdominal pressure changes related to retches or vomits, it was important to obtain a significant number of emetic events not only during the delayed phase but also during the early phase. Automated analysis of the signals was performed using ecgAUTO (version v3.2.0.1.) software modified in collaboration with Emka. This software provides a considerable number of parameters including the number of large or short peaks, the amplitude of the peak (maximum and minimum), the number of peaks recorded per series, the area under the curve and the latency to first event. The software is able to analyze a 72-hour recording session in a few minutes as compared with several hours by a technician using manual/visual detection. After the analysis has ended, each individual line with the corresponding series of emetic events can be reviewed and any undesired event (for example presence of an artifact) can be excluded.

The original version of ecgAUTO software is already used in safety pharmacology studies compliant with Good Laboratory Practice (GLP). The modified version used in the present study possesses therefore the considerable advantage that it can be readily validated for GLP-compliance.

In parallel to telemetry recordings, animal behavior was recorded on video. In addition, ferrets were directly observed by highly-trained technicians over a 3-hour period, starting immediately after the cisplatin challenge.

Because of cisplatin toxicity, the animals were sacrificed immediately at the end of the 72-hour recording period.

### 2.4. Expression and analysis of the data

#### 2.4.1. Visual detection of emesis recorded by observation

Each ferret was observed by technicians who recorded the number of retching and vomiting events over the 3-hour recording period. Retching was defined as a rhythmic respiratory movement against a closed glottis, while vomiting was defined as a forced expulsion of upper gastrointestinal contents.

#### 2.4.2. Manual detection of emesis recorded by telemetry

The pressure signals of each ferret were visually inspected off-line by a trained technician who manually reported the number of emetic events (retches or vomits) over the 72-hour recording period. The video was used in case of doubt and/or loss of signals (e.g. movement of the animal interfering with and/or inducing a disruption of the signal for instance). An emetic event was recognized as an isolated contraction or a sequence of contractions of the abdomen inducing an increase of abdominal pressure signals (see Fig. 1). After a review of a large number of signals, the following criteria were defined for each animal: the width of the peak, related to the contraction duration (<1500 ms), the time between peaks within a series (3 s) and the amplitude of the peak (to be adjusted for each ferret). This is consistent with the conclusions of Rudd and Naylor (1996) who suggested that episodes of retching and/or vomiting should be considered as separate when the interval between retches and/or vomits exceeds 5 s.

#### 2.4.3. Automated detection of emesis recorded by telemetry

As for manual detection, the following criteria were defined for each animal: the width of the peak (<1500 ms), the time between peaks within a series (3 s) and the amplitude of the peak (to be adjusted for each ferret).

The analyzed data can be then exported in an Excel® spreadsheet.