Acetaminophen absorption test: a method for the evaluation of gastric emptying in cynomolgus monkey

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Introduction
Absorption and systemic exposure of orally administered new candidate drugs are often closely related to gastric emptying kinetics. In addition, gastric-oesophageal reflux or emesis, which are sometimes observed during non-clinical toxicity studies, may sometimes be related to a delayed gastric emptying, rather than a direct systemic effect of the drug.

The purpose of the present study was to establish the acetaminophen intestinal absorption test in cynomolgus monkeys (Macaca fascicularis) as a suitable biomarker for routine evaluation of gastric emptying in our laboratory.

The rationale for use of this methodology is based on the fact that acetaminophen is not absorbed from the stomach but rather from the small intestine and therefore the kinetics of the appearance of acetaminophen in blood after single oral administration is considered as an indirect method for determining the rate of gastric emptying (Murphy et al., 1997, Heading R. C. et al., 1973).

Materials and methods

Animal model

Four cynomolgus monkeys weighing 3.6 to 4.8 kg (two males and two females, Noveprim, Mauritius) were used in the present study.

Prior to inclusion in the study, the animals were acclimated to the study conditions for a period of at least 7 days and blood biochemistry and hematology values were checked to ensure that the animals were in good health. On the days of treatment, the animals were deprived of food approximately for 14 hours before dosing, but they were not deprived of water. Food was given no sooner than 6 hours after dosing.

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Treatments

All animals were given 60 mg of acetaminophen by the oral route at 5 mL/kg. Immediately thereafter, all animals received an intravenous injection of 2 mg/kg of the antiemetic and gastroprokinetic agent metoclopramide, or the same volume of the vehicle (0.9%NaCl) at 1 mL/kg.

Plasma sampling

To evaluate plasma concentrations of acetaminophen, blood samples (approximately 1 mL) were taken immediately before administration and then, 10, 20, 30, 45, 60, 90, 120, 240 and 360 minutes after dosing. Samples were centrifuged within 30 min after collection at 3500 g for 10 minutes at 4°C and two aliquots of plasma (at least 200 µL each) were kept frozen in individual tubes at -20°C until analysis.

Plasma processing

The plasma samples (50 µL) were supplemented with internal standard (gabapentin, 10 µL of a solution of 5 µg/mL) and proteins were precipitated (acetonitrile, 300 µL). After centrifugation, the supernatant was evaporated, reconstituted with mobile phase and analyzed by high-performance liquid chromatography (Phenomenex Luna PFP, water/methanol 90/10 v/v + 0.1 formic acid) and tandem mass spectrometry (m/z 152.1-110.1 and 172.2-154.2). Calibration lines were linear (1/x2) with correlation coefficient higher than 0.990. An example of an acetaminophen chromatogram is presented in Figure 1.

Pharmacokinetic parameters

The main pharmacokinetic parameters of acetaminophen were determined according to a standard non compartmental method using WinNonlin software version 5.01 (Pharsight Corporation). Specifically, the maximum plasma concentration (Cmax) and the time to peak concentration (Tmax) were read directly from the concentration/time plot, whereas the area under the concentration/time curve from time 0 to the last time-point with quantifiable data (AUC0-t) were calculated according to the linear trapezoidal rule.

The constant of absorption (Ka) and the half-life of absorption (t1/2a) were estimated by a one-compartment model using WinNonlin software.

Statistical analyses

Statistical analyses were performed using SAS software version 9.2 (SAS Institute Inc.) two way (time and treatment) analysis of variance (ANOVA) for repeated measurements followed by a one-way ANOVA and Dunnett test at each time-point.

Discussion and conclusions

The results of the present study demonstrate that administration of the antiemetic and gastroprokinetic agent metoclopramide, significantly stimulated gastric emptying in cynomolgus monkeys as shown by the marked increase of Cmax, associated with a reduction in Tmax.

These results support the utility of the acetaminophen absorption test in the cynomolgus monkey for routine evaluation of gastric emptying in non-clinical development.

Results

The mean maximum plasma concentration (Cmax) of acetaminophen was reached 90 min. after administration (Tmax) of the vehicle, while it reached 6333 ng/mL 30 min. after administration of metoclopramide. This difference did not achieve statistical significance (p=0.052) (Figure 2). Acetaminophen AUC0-t values were 6039 and 8399 ng.h/ml after treatment with vehicle and metoclopramide, respectively (Figure 3).

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These results support the utility of the acetaminophen absorption test in the cynomolgus monkey for routine evaluation of gastric emptying in non-clinical development.

References

• Murphy et al., Opioid-induced delay in gastric emptying. Anesthesiology. 87: 762-770, 1997.