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Improved use of the [^{13}C]octanoic acid breath test as intra-individual parameter to study the effect of a prokinetic drug on gastric emptying in preterm infants with oral feeding intolerance

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Abstract

The [^{13}C]octanoic acid breath test was used for the measurement of differences in gastric emptying in preterm infants for the evaluation of pharmacological therapy. In order to perform a good intra-individual comparison of the gastric emptying in preterm infants under non-standardisable test conditions, we adjusted $t_{1/2}$ for variations in non-recovered label (=label retention) and introduced an ''effective half $^{13}CO_2$ breath excretion time'' $t_{1/2eff}=t_{1/2}/m$ expressed as min per percentage of the cumulative dose recovered. In a pilot study, we investigated the action of the gastrointestinal prokinetic drug cisapride on gastric emptying in seven premature infants, of whom four suffered from gastric stasis and three had constipation. The postnatal age and weight at the start of treatment ranged from 15 to 64 days and from 815 to 1635 g, respectively. All infants received the standard formula for premature infants (Nenatal, Nutricia). Cisapride was administered orally 0.2 mg/kg, four times daily. The changes in gastrointestinal motility were studied using the total bowel transit time of carmine red. After 7 days of treatment in all children, the gastric emptying coefficient and the half $^{13}CO_2$ breath excretion time adjusted for label retention were improved ($n=7$, the gastric emptying coefficient range before treatment was 1.69–3.34 (mean 2.59±0.80) and after treatment it was 2.79–3.76 (mean 3.28±0.30); the half $^{13}CO_2$ breath excretion time adjusted for label retention range before treatment was 3.0–14.7 min/% dose (mean 7.0±5.0) and after treatment 2.6–4.0 min/% dose (mean 3.1±0.6). The total bowel transit time was only slightly improved in two patients ($n=7$, mean total bowel transit time before: 23.7 h compared to mean total bowel transit time after 7 days of treatment: 35.5 h). Side effects during cisapride treatment were not seen. We conclude that in premature infants cisapride is effective in shortening gastric emptying time and reducing gastric stasis; the therapeutic role in constipation has to be further investigated.

Keywords: [^{13}C]octanoic acid

1. Introduction

Gastrointestinal motility consists of a complex, highly coordinated, neuromuscular process [1–3].

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Histological and ultrastructural studies show the presence of enteric nerves and smooth muscle cells within the wall of the human intestine during the second trimester, and by 22 weeks of gestation the small intestine is histologically mature [4]. Clinical experience shows that the introduction of enteral feedings to preterm infants is often met with feeding intolerance characterized mainly by gastric retention. Gastric stasis is known to increase the risk of gastro-oesophageal reflux and subsequently the risk of pulmonary aspiration and apnoea [5,6].

Several methods of measurement exist for the determination of the rate of gastric emptying in adults and children which comprise radioscintigraphy (e.g. [7]), impedance tomography, antroduodenal manometry, magnetic resonance imaging and several invasive techniques (e.g. [8,9]). However, for small children the use of a practical, non-invasive test without radiation burden seems to be the most likely. Next to the ultrasonography the [^{13}C]octanoic acid breath test (OBT) is a good alternative for the measurement in small children [10–12]. We describe an extension of the OBT for the use with premature infants for which completely standardized test meals were not possible. We demonstrate our improved method by a pilot study on the effect of the prokinetic drug cisapride on gastric emptying. Cisapride (Prepulsid®, Janssen Pharmaceutical) is a substituted piperidinyl benzamide without antidopaminergic or direct cholinomimetic effects. Cisapride restores or facilitates motility throughout the length of the gastrointestinal tract. It also increases the lower oesophageal sphincter pressure, improves oesophageal clearance, stimulates gastric emptying of contents and accelerates small intestinal and colonic propulsion [13].

There is substantial literature that shows cisapride to be efficacious in the treatment of clinical conditions that are likely to result from defective motor function at different levels of the digestive tract. Most of the pharmacokinetic data are, however, related to adults and children) (e.g. [14–19]). Studies on the role of cisapride on gastrointestinal motility in preterm infants are very limited [6,20,21]. Moreover, the effect of cisapride on constipation in preterm infants is not clear. Therefore, the present pilot study was undertaken to study the effect of cisapride on gastric emptying with the non-invasive extended OBT.

2. Experimental

2.1. Patients and methods

Seven preterm infants of whom four suffered from gastric stasis and GE-reflux and three with constipation were enrolled in the study. Gestational ages ranged from 27 to 29 weeks and birth weights from 740 to 1495 g. The postnatal ages and weights at the start of the study ranged from 15 to 64 days and from 815 to 1635 g, respectively.

In all infants systemic diseases and organic causes of gastrointestinal motility disorders were assumed to be absent on the basis of normal biochemical tests and abdominal X-ray. Cardiac and cerebral diseases were ruled out on the basis of ultrasonography. Two infants were ventilated, two infants had nasal CPAP (continuous positive airway pressure) and another two had a low-flow oxygen support by a nasal canula, one infant had no ventilatory support.

Cisapride was administered orally 0.2 mg/kg q.i.d. 15–30 min prior to a meal, for 7 days. All infants had been stabilized on parenteral feeding when they were started on enteral feeding. All infants received standard formula for premature infants (Nenatal, Nutricia) gavage feeding, in a 1–3-h frequency based on their age and actual weight. ECG lead I, II or III was continuously monitored in all treated infants.

2.2. Total bowel transit time

Total bowel transit time (TBTT) was studied before and after 7 days of cisapride treatment using the passage time of carmine red. Therefore, all infants received a carmine red containing meal. Baseline TBTT was established with the first passage of the carmine red.

2.3. [^{13}C]octanoic acid breath test

Gastric emptying was measured by means of the non-invasive [^{13}C]octanoic acid breath test (OBT) [22–25], which has been validated for adults and preterm infants [12,25–32].

We applied the methodology of the OBT as adapted by Veereman-Wauters et al. [10]. For practical reasons we estimated the CO_2 production of this group of preterm infants at 18.3 mmol/kg/h, based upon data from previous studies in similar groups of

patients [10,30], and used this as a constant value throughout the mathematical analyses (It was shown that in similar groups of preterm infants the effective total CO_2 production measured by indirect calorimetry could be well estimated by a constant value. The differences between preterm children for the mean CO_2 production (C.V.<15%) during the test period are relatively small and will most unlikely account for a large variation in the ''half $^{13}CO_2$ breath excretion time''. The relief for the preterm child for not measuring by indirect calorimetry seemed worth the plausible concession for not measuring the CO_2 production by indirect calorimetry).

All infants received a test meal mixed with low and stable natural ^{13}C-abundance mixed with 50 μl of [^{13}C]octanoic acid ([1-^{13}C] 99% AP, Isotec, Ohio, USA) and 1 g of polyethylene glycol 3350 (it showed that feeding the test meal without the labelled octanoic acid did not affect baseline breath air values). They received the test meal at the time of their usual first morning feeding, allowing for 3 h of fasting. Breath samples were collected using a nasal prong (Sherwood CH 6) in case the patient was not mechanically ventilated. Otherwise, samples were collected using a connection securely attached to the outflow source of the ventilator of the ventilated patient, in which case the patient was not disconnected during sampling. Five minutes before the test meal five breath samples were collected at basal conditions. After feeding the test meal, breath samples were taken every 5 min during the first half hour and every 15 min during the next three and a half hours. The OBTs were performed 1 day before administration of cisapride and after 7 days of cisapride treatment. Cisapride was administered 2 h before the test meal.

2.4. Isotope ratio mass spectrometry

Measurement of ^{13}C-enrichment in exhaled air was performed with an isotope ratio mass spectrometer in the dual inlet mode (VG Optima, Micromass, Manchester, UK) using cryogenic cleanup. Ions were generated by electron impact ionization with electron energy of 100 eV. The accelerating voltage and magnetic current were 3.5 kV and approximately 3.3 A, respectively. Ion currents were measured for m/z 44, 45 and 46 using a set of three Faraday cups. The $^{13}CO_2$-enrichment was measured against a reference carbon dioxide gas, calibrated relative to Pee Dee Belemnite (PDB); ^{13}C content was calculated, using standard convention, as δ^{13}C (‰) values. The geometric mean of the five baseline breath air values was used for $t=0$ min.

2.5. Statistical analysis

Data were reported as ''mean±SD''. Differences in gastric emptying coefficient (GEC), ''half $^{13}CO_2$ breath excretion time'' ($t_{1/2}$), ''half $^{13}CO_2$ breath excretion time adjusted for label retention'' ($t_{1/2eff}$), TBTT, before and after 7 days of cisapride treatment were analysed with the non-parametric Wilcoxon Signed Ranks Test (2-tailed).

3. Results and discussion

In our pilot study to investigate the effect of the prokinetic drug on gastric emptying in premature infants we observed large inter- and intraindividual variation in the cumulative percentage of the dose recovered (when time is infinite) (range=6–62%; mean=31±15%; $n=13$; the maximum ''δ^{13}C over baseline'' (DOB) values: range=20–178‰; mean=114±50‰; $n=13$) and thus, in the rate in which the percentage of the dose was recovered. Nevertheless, the shape of all excretion curves [(i) %dose/h vs. time (h) and (ii) cumulative %dose vs. time (h)] could be well described by the formulas developed by Maes and Ghoos [22,24,28] (e.g. Fig. 1).

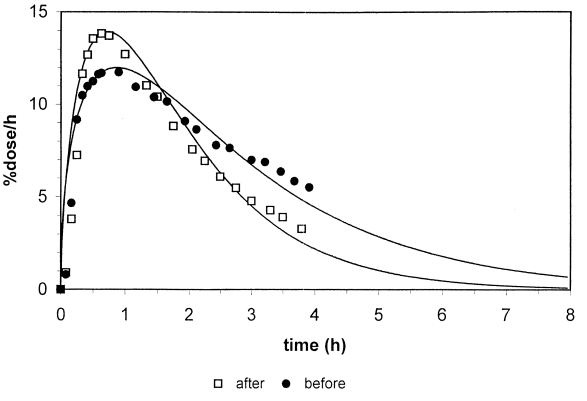

Fig. 1. Excretion curves of a ventilated patient (1200 g; dose 50 μl) before and after medication during the [^{13}C]octanoic acid breath test.

Typical $^{13}CO_2$ excretion curves, expressed as %dose/h vs. time (h), can be fitted mathematically by two formulas: (a) $y = mk\beta\ e^{-kt}(1 - e^{-kt})^{\beta-1}$; (b) $y = at^b\ e^{-ct}$. From these formulas, GEC and $t_{1/2}$ can be derived where GEC is a function of ''a'' and $t_{1/2}$ is a function of ''k'' and ''β'', if derived from the first formula or $t_{1/2}$ is a function of ''b'' and ''c'' if derived from the second formula. The results by both formulas are similar. Since it is still under debate whether it is justified to adjust $t_{1/2}$ to related data obtained from scintigraphy (with adults), we used $t_{1/2}$ without the correction for time delay. Therefore, the term ''gastric half emptying time'' was replaced by the term ''half $^{13}CO_2$ breath excretion time'' and was calculated as $t_{1/2} = (-1/k)\ln(1 - 2^{-1/\beta})$*60 expressed in min instead of $t_{1/2} = [(-1/k) \times \ln(1 - 2^{-1/\beta})$*60 − 66]/1.12. The expected correlation between the mathematically derived parameters GEC and $t_{1/2}$ was, however, completely absent: $r = -0.07$ ($n = 13$).

Previous studies suggested that the OBT could be a useful tool in intraindividual comparison studies of gastric emptying [26,27]. A relatively large variability was, however, observed for the OBT in the interindividual comparison studies of gastric emptying in both adults and preterm infants [27,10]. The variability in the latter group was mainly attributed to a lack of possibilities to standardise the test conditions. Obvious causes for the lack of correlation between GEC and $t_{1/2}$ in our pilot study, such as vomiting after administration of the substrate, incomplete administration of the test meal and bad breath sampling, etc. could be excluded (breath sampling was performed by a trained research nurse very familiar with all the pitfalls in breath sampling under the study conditions; breath samples were analysed for the amount of collected CO_2 and the presence of contamination's. If bad sampling or incomplete administration of substrate was observed the patient was excluded from the study.). We therefore approached the problem differently.

On the basis of the mathematical formulas $t_{1/2}$ is not affected by the amount of octanoic acid absorbed (or $^{13}CO_2$ produced); whether 1% or 99% of the dose is absorbed, based on data measured the same result for $t_{1/2}$ will be found and thus gastric emptying will be diagnosed as being the same. It seemed, however, plausible that different types of gastric emptying can affect the possibility of absorbing octanoic acid effectively in the duodenum. Hypothetically, fast gastric emptying accompanied with complete absorption of all octanoic acid can result in a larger $t_{1/2}$ value than a slow gastric emptying with a minimal and incomplete absorption. Therefore, intraindividual comparison seemed justified only if the coefficient of variation for the ^{13}C recovery in the same subject is small (e.g. 20% Ghoos et al., 12% Choi et al). In these cases $t_{1/2}$ estimates the time necessary for the first half of total substrate recovered (when time is infinite) to be exhaled as $^{13}CO_2$. It is, however, not a gastric emptying rate and it is a valuable parameter by virtue of completely standardised test conditions with low coefficients of variation.

If standard conditions are very difficult to maintain, as it is for preterm infants with relatively large variations in metabolism, (intra- and interindividual) variations in label recovery could be expected and conclusions from data unsusceptible to these large variations become questionable. By introducing a parameter that is adjusted by label recovered effectively, it seemed possible to compare intraindividual data even if the variations in recovery were large. By dividing $t_{1/2}$ by m (percentage of the dose recovered when time is infinite) it becomes a parameter which describes a sort of mean ''half $^{13}CO_2$ breath excretion time'' necessary for a percent of the dose to be recovered effectively. This $t_{1/2\text{eff}}$ is a mathematical parameter rather than a real physiological parameter related by indirect processes to the rate of gastric emptying. Linear regression showed a good correlation between GEC and $t_{1/2\text{eff}}$ ($r = -0.89$, $n = 13$), between GEC and $\ln(t_{1/2\text{eff}})$ ($r = -0.91$, $n = 13$) (see Fig. 2a, b) where correlation between GEC and $t_{1/2}$ was lacking (although a good correlation was expected in respect of data presented by Maes [24]).

The good correlation between GEC and $t_{1/2\text{eff}}$ suggests therefore that $t_{1/2\text{eff}}$ is a more appropriate parameter than $t_{1/2}$ to study this group of preterm children. It has, however, to be considered that the GEC values are affected by the amount of octanoic acid effectively absorbed. Therefore, it seems necessary to study in which way the rate of gastric emptying can affect absorption of octanoic acid in ill preterm infants to enhance the usefulness of the OBT for studying these patients.

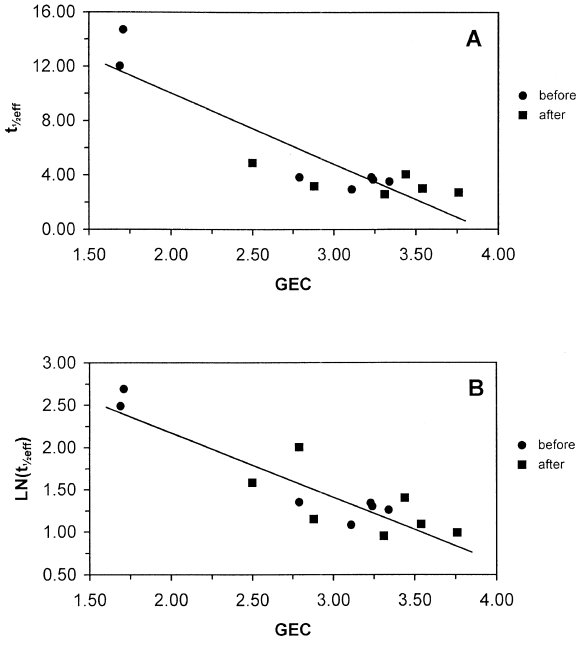

Fig. 2. Correlation between GEC and $t_{1/2eff}$; $r=-0.89$ for $n=13$ tests (panel A) and correlation between GEC and ln($t_{1/2eff}$); $r=-0.91$ for $n=13$ tests (panel B).

Patients data are shown in Table 1. The values representing GEC, $t_{1/2}$, $t_{1/2eff}$ and TBTT before and after 7 days of cisapride treatment are shown in Table 2. In all infants gastric stasis was improved within 48 h and vomiting decreased within 24 h and disappeared 72 h after starting treatment. The gastrointestinal motility expressed by the TBTT of carmine red was only slightly but not significantly improved in two patients (range before treatment: 10–38 h; mean 23.8±10.8; range after 7 days of treatment 17–58.5 h, mean 35.6±16.6) but not in the three infants with constipation (Table 2). We did not find a plausible hypothesis that explained the observed clinical improvements in terms of the measured TBTT values based on red carmine. This was probably due to: (i) the large time interval between start and end of red carmine production (with large C.V.'s) which made interpretation of the mean values unreliable and (ii) there is no direct relation between TBTT and ease of defecation. Therefore, we concluded that use of the red carmine test for this type of patient is at least questionable.

Table 1
Values of gestational age (GA), postconceptional age (PCA) respiratory conditions and diagnosis of preterm patients in the pilot study

Patient	GA	PCA	Respiratory support	Diagnosis
A	27+4	28+5	Low-flow O_2	Constipation
B	29+3	32+5	NCPAP	GE reflux
C	29+4	35+4	SIPPV	Constipation
D	27+6	30+2	SIMV	GE reflux
E	29+2	31+4	–	Constipation
F	29+6	33+2	NCPAP	GE reflux
G	29+6	34+3	Low flow O_2	GE reflux

The GEC measured according to Veereman-Wauters et al. [10] improved after 7 days of medication: range 2.79–3.76 (mean 3.28±0.30) compared to the GEC measured before treatment: range 1.69–3.34 (mean 2.59±0.80); the significance level of the Wilcoxon signed ranks test was 0.028, indicating a significant difference between the paired values (paired *t*-test showed also a significant difference: $P=0.035$); Normal values according to Veereman-Wauters range 2.7–3.4 [10].

The observed significance level with the Wilcoxon signed ranks test for the measured $t_{1/2}$ values was very large (0.345), rejecting the hypothesis of a significant difference between the paired values before and after treatment. The observed significance level for the $t_{1/2eff}$ values was 0.075. Taking into account the small sample size (six pairs), it suggests a real difference between the paired values before and after treatment; $t_{1/2eff}$ showed an improvement in five of six cases. Since the sample sizes before and after treatment are small and the variability is large, even the apparent substantial differences were likely not to be detected by using the paired *t*-test. Therefore, analysis of $t_{1/2}$ and $t_{1/2eff}$ was more appropriate by the non-parametric Wilcoxon signed ranks test, indicating the necessity to use patients as their own reference.

In preterms, the problem of normal gastric emptying versus gastric stasis or intolerance of feeding is very complex. Feeding intolerance, characterised by gastric retention, is dependent on gestational maturity and postnatal age. Poor enteral feeding and subsequent failure to thrive is a well-known problem in premature neonates. The reduction of gastric stasis in order to institute enteral feeding sooner will also lead to a consequential reduced use of catheters and

Table 2
Values of the gastric emptying coefficient (GEC), half $^{13}CO_2$ breath excretion time ($t_{1/2}$, min) and effective half $^{13}CO_2$ breath excretion time ($t_{1/2eff}$, min/%cum dose) based on the OBT and the total bowel transit time [TBTT (in h)] using carmine red of patients in the pilot study before and after treatment

Patient	GEC		$t_{1/2}$		$t_{1/2eff}$		TBTT	
	Before	After	Before	After	Before	After	Before	After
A	2.50	2.79	115.6	114.1	4.9	3.8	18–54	21–21
B	1.71	3.31	92.8	118.5	14.7	2.6	36–44	49–62
C	3.11	3.76	126.7	87.4	3.0	2.7	34–62	58–75
D	1.69	2.88	112.0	197.2	12.0	3.2	38	17–31
E	3.34	3.44	112.2	101.0	3.5	4.0	29–77	47–96
F	–	3.24	–	122.2	–	3.7	10–58	36–43
G	3.23	3.54	95.8	99.8	3.8	3.0	21–31	20–26
Mean	2.6	3.3	109.2	120.0	7.0	3.3	23.8	35.6
SD	0.8	0.3	12.7	36.1	5.0	0.6	10.8	16.6

of total parenteral nutrition [5,6]. In our study, the administration of cisapride for a short duration (7 days) to premature infants was well tolerated; no side effects, i.e. no disturbances on ECG registration were observed. During treatment, gastric stasis and GE reflux disappeared and oral feeding was well tolerated. These results were confirmed by the improvement in GEC and $t_{1/2eff}$.

In our study, the neonates had variable feeding schedules based on their age, weight and feeding tolerance. After absorption, [^{13}C]octanoic acid will be oxidised to CO_2 or transformed by chain elongation into a long chain fatty acid, resulting in triglyceride storage. In the latter case and when absorption does not occur, the ^{13}C-label does not appear in the exhaled air and large differences in the amount of label recovery may occur. Based on the assumption that the variation in data resulted mainly from variations in feeding schedules and differences in the anabolic/catabolic conditions, $t_{1/2}$ was divided by the percentage cumulative dose if time was infinite ($=t_{1/2eff}$) which resulted in a linear relationship between GEC and $t_{1/2eff}$.

In contrast to our study, McClure et al. [33] recently showed that cisapride treatment in preterms delays gastric emptying determined by using ultrasonography on day 3 of treatment by serially measuring the change in gastric antral cross-sectional area following a feed. However, this method of studying gastric emptying differs greatly from ours and it is difficult to compare their results with our findings. Moreover, their method for calculating half gastric emptying time (the time taken for the antral cross-sectional area (ACSA) to decrease by half of the maximum change seen during gastric filling) seems to be influenced by the total amount of the bolus feeding. However, no correction was made for the large variation in the amount of bolus feeding in the patients studied.

4. Conclusion

It is plausible that gastric emptying of preterm infants under non-standardisable test conditions can still be studied using the OBT, with GEC and an ''effective half $^{13}CO_2$ breath excretion time'' $t_{1/2eff}$ as test parameters and patients as their own reference; interindividual comparison based upon $t_{1/2}$ did not show reliable results; the red carmine test in this group of preterm patients did not seem to be a usable tool for measuring TBTT.

In view of the risks of complications of gastric stasis and gastro-oesophageal reflux as well as the drawbacks of late enteral feeding in premature infants, treatment with cisapride in this group of patients shows a tendency to be effective.

A larger study is justified to further evaluate the effectiveness and safety of cisapride in this group of patients and is in progress.

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