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Research Article

Critical evaluation of the chemical standardization procedure for measuring gastric emptying of solids

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Summary

The purpose of this study was to evaluate the baking process of yolk spiked with octanoate to measure gastric emptying rate of solids. [1-^{11}C]octanoate was produced by the reaction of [^{11}C]CO_2 with heptyl magnesium bromide in tetrahydrofuran (THF), followed by purification with HPLC. The decay corrected radiochemical yield ranged from 24 to 38% (5.9–9.8 GBq EOS, synthesis time: 25 min; specific radioactivity $\sim$90 GBq μmol^{-1}). To check the evaporation of [1-^{11}C]octanoate during the baking process of yolk, [1-^{11}C]octanoate or potassium [1-^{11}C]octanoate, respectively, was added. An important fraction of the acid evaporated while for the potassium [1-^{11}C]octanoate $<$10% disappeared. *Conclusion*: potassium (1-^{13}C)octanoate is a better tracer than (1-^{13}C) octanoate to study gastroenterological phenomena.

Key Words: [1-^{11}C]octanoate; PET; gastric emptying

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Introduction

Gamma scintigraphy is generally accepted as a reference method (*gold standard*) for the determination of gastric emptying.[1–3] However, the method requires the availability, competent operation, and prolonged occupancy of complex and expensive equipment (γ-and/or SPECT-camera). Moreover, the use of penetrant γ-ray emitters (70–300 keV) makes those techniques less suitable for repeated applications in the same patient.[1] These considerations led to the development of alternative methods, which are easier to perform by a laboratory technician, are comfortable for the patient and should correlate well with the result obtained by γ-scintigraphy. Ghoos *et al.*[4] developed and validated a breath test for the measurement of gastric emptying of solids, labelled with (1-^{13}C)octanoate (100 mg). The rationale of the test is based on the disintegration of the labelled solid phase of a test meal with subsequent absorption and oxidation of (1-^{13}C)octanoate to (^{13}C)carbon dioxide, which occurs once the meal reaches the duodenum.

These authors elaborated out a standard procedure to prepare a test meal consisting of a scrambled egg with the yolk doped with the sodium salt of [1-^{14}C]octanoic acid (DuPont, NEN Research, Boston, MA).

To evaluate the baking procedure as described by Ghoos *et al.*,[4] the synthesis of [1-^{11}C]octanoate was carried out starting from [^{11}C]CO_2. ^{11}C has the advantage over the other carbon isotopes that it can be easily detected with a calibrated proportional counter (Capintec®). The ^{11}C-labelled octanoate is (bio)chemically identical with the naturaly occurring ^{12}C and ^{13}C analogues.

Based on data of previous work[5] and the extended study of Kihlberg *et al.*,[6] we adapted the experimental circumstances to perform the synthesis in the remotely controlled unit for [methyl-^{11}C]thymidine.

Experimental

Materials and methods

Synthesis of [1-^{11}C]octanoic acid. The [^{11}C]CO_2 was, after the irradiation (40 min, 15–17 μA, yield 59 GBq) trapped in a stainless-steel coil (flow rate 4.0 l min^{-1}) immersed in liquid argon (−186°C). $^{11}CO_2$ was brought at room temperature and transferred by helium carrier gas (dried over P_2O_5; flow rate 7 ml min^{-1}) to a vessel containing heptyl

magnesium bromide in THF (250 µl, 0.5 M, −20°C). Immediately after the transfer of the activity, 1 ml of a methanol/water solution (70/30) was added to destroy the excess of Grignard reagent and to stop further reaction to the formation of [^{11}C]diheptyl keton. With a peristaltic pump, the reaction mixture was brought into the HPLC loop (0.5 ml). For the purification, HPLC was applied on a reversed-phase semi-preparative column (RSIL C_{18}, HL $25 \times 1\,cm^2$, 10 µm) using methanol/water (70/30 v/v) as *eluent* at a flow rate of 3 ml min^{-1}. γ-ray (ionization chamber) and RI detection were used for the identification of the different components in the HPLC effluent. The radioactive fraction, corresponding with the retention time of octanoate, was filtered on a Millipore® membrane (0.22 µm pore size) and collected into a 10 ml sterile, apyrogenic vial. [1-^{11}C]octanoate was baseline separated from the chemical and radiochemical side products (Scheme 1).

$$CH_3(CH_2)_6Br \xrightarrow[THF]{Mg} CH_3(CH_2)_6MgBr \xrightarrow[2:\ CH_3OH/water]{1:\ ^{11}CO_2} CH_3(CH_2)_6{}^{11}COOH$$

Scheme 1. Synthesis of [1-^{11}C]octanoate

Baking process. [1-^{11}C]*octanoate*. To 2 ml (2.0–2.1 g) egg yolk, 0.1 ml of H_2O solution containing 9 MBq (2.5 mCi) of [1-^{11}C]octanoate was added and manually homogenized for 2 min in a glass beaker of 25 ml. Previously, tests were done to control the homogeneous partition of the activity in the yolk before starting the backing process. After measuring the total activity with a Capintec® detector (calibrated ionization chamber), the yolk was baked at a temperature of 85°C. During the baking process, there was a constant Ar flow of 20 ml min^{-1} to remove the evaporated liquid. At fixed time intervals (0, 5, 10,. . ., 30 min), the remaining activity in the egg yolk was measured with the Capintec® detector, corrected for decay and plotted *Versus* time.

Potassium [1-^{11}C]*octanoate*. To obtain potassium[1-^{11}C]octanoate, 10 µl of 0.1 M KOH solution was added to the purified [1-^{11}C]octanoate. The same baking protocol, as described previously for the [1-^{11}C]octanoate, was applied.

In three additional experiments, 100 mg of (1-^{13}C)octanoate carrier or 120 mg potassium (1-^{13}C)]octanoate resp. was added simultaneously with the radioactivity to the egg yolk, homogenized and the procedure executed as described above.

Results and discussion

Synthesis of [1-^{11}C]octanoate

A decay corrected radiochemical yield ranging from 24 to 38% was obtained (5.9–9.8 GBq EOS, synthesis time: 25 min; specific radioactivity $\sim$90 GBq μmol^{-1}). Instead of diethyl ether/heptane (50/50) as solvent as described by Kihlberg *et al.*,[6] in our technical set-up, THF gave more reproducible results. Quality control of the final product [1-^{11}C] octanoate using HPLC indicated that the radiochemical purity was at least 99%. The chemical structure and purity were confirmed by ^{1}H-NMR on a bulk synthesis sample. Therefore, during trapping the [^{11}C]CO_2 in the Grignard solution, simultaneously 10 μmol CO_2 was added and the synthesis was carried out under the same circumstances.

Additional quality control of the purified radiopharmaceutical (analytical GC/FID, T gradient 150–200°C, 10°C min^{-1}) as described by Kihlberg *et al.*[6] confirmed our data obtained that the carrier amount was $<$0.1 μmol and radiochemically pure.

Homogenization and baking process

In order to enable the exact quantification of the amount of [1-^{11}C] octanoate, we standardized the duration of yolk homogenization and mixture, and especially the duration and the temperature of the baking process. The results of the baking experiments with [1-^{11}C]octanoate ($n=5$) res. potassium [1-^{11}C]octanoate ($n=4$) are visualized in Figures 1 and 2.

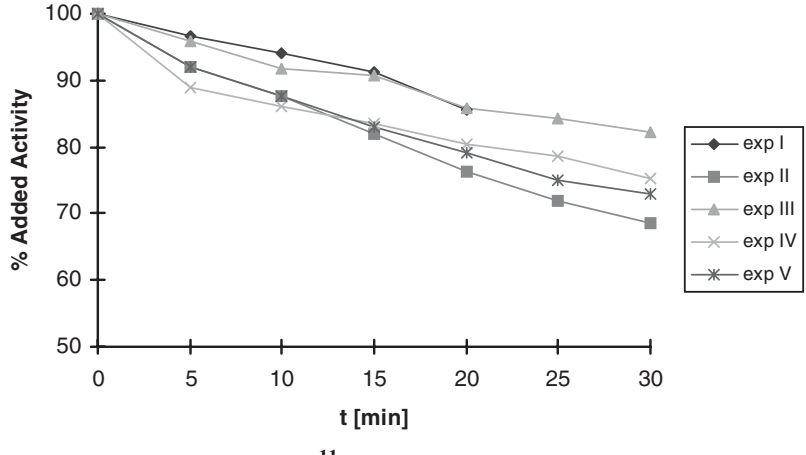

Figure 1. Disappearance of [1-^{11}C]octanoate during the baking process

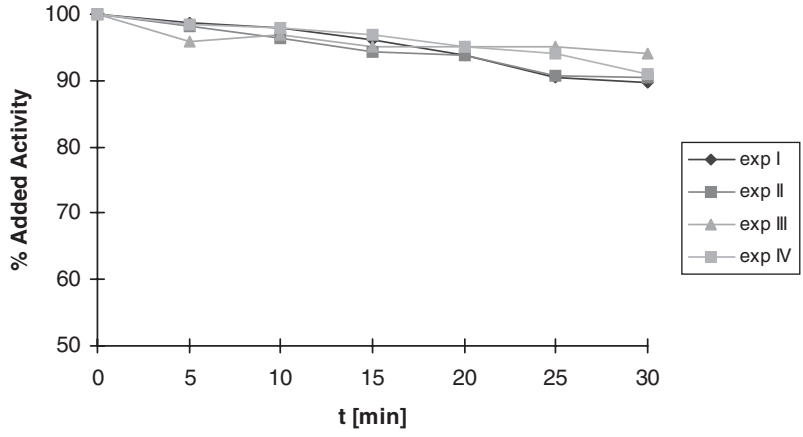

Figure 2. Disappearance of potassium [1-^{11}C]octanoate during the baking process

During the baking procedure, a steam distillation took place resulting in the evaporation of the [1-^{11}C]octanoate at a temperature far below the theoretical boiling point of 237°C. Evaporation of the moisture resulted in a decrease of 30–45% of the total weight of the original egg yolk. Simultaneously, there was an important decrease of the [1-^{11}C]octanoate activity (Figure 1). Due to the completely different chemical and physical properties of potassium [1-^{11}C]octanoate versus [1-^{11}C]octanoate, no substantial decrease of potassium [1-^{11}C]octanoate activity took place during the baking procedure (Figure 2).

No substantial difference was noticed between the results of the baking process with or without adding (1-^{13}C)octanoate carrier.

Conclusion

The baking process for the preparation of octanoate doped egg yolk, as used for the determination of gastric emptying,[4] resulted in the evaporation of a substantial fraction of the administered (1-^{13}C)octanoate. Absolute quantification of the gastric emptying rate for solids as fraction of the % administered (1-^{13}C)octanoate dose by measuring the exhaled $^{13}CO_2$ might impose problems since the ingested amount of (1-^{13}C)octanoate is unknown. Relative quantification is still possible.

Although the absolute amount of administered (1-^{13}C)octanoic acid has not to be known for the calculation of the gastric emptying time $t_{1/2}$, it is necessary for the calculation of other parameters such as the gastric

emptying coefficient (GEC). Diagnostic change on gastric emptying, pre and post administration of a pharmaceutical, for the same patient requires absolute quantification of the expired $^{13}CO_2$. Knowledge of the exact amount remaining after baking is only possible when using the salt. We therefore propose to add octanoate (sodium or potassium) rather than acid to the egg yolk when gastric emptying determinations are performed.

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