Biodistribution and Dosimetry of Carbon-11-Methoxyprogabidic Acid, a Possible Ligand for GABA-Receptors in the Brain

Patrick Santens, Filip De Vos, Hubert Thierens, Danny Decoo, Guido Slegers, Rudi A. Dierckx and Jacques De Reuck
PET Center, University Hospital and University of Gent; Department of Neurology, University Hospital; Departments of Radiopharmacy and Biomedical Physics, University of Gent; and Department of Nuclear Medicine, University Hospital Gent, Belgium

Carbon-11-methoxyprogabidic acid (^{11}C-MPGA) was recently synthetized as a possible ligand for PET studies of gamma-amino-butyric acid (GABA) receptors in the brain. The data for human absorbed dose estimates are calculated based on the biodistribution of ^{11}C-MPGA in mice and humans. **Methods:** Eighteen mice were killed at preset time intervals after an intravenous bolus injection of 3.7 MBq (100 μCi) ^{11}C-MPGA. Time-activity curves were reconstructed for several organs. Three healthy men each had whole-body PET scans after an intravenous bolus injection of 37 MBq (1 mCi) to determine activity in the critical organs. Animal data were fitted into these human findings to calculate residence times, and the MIRDOSE 3 protocol was used to calculate the radiation absorbed dose. **Results:** Animal studies demonstrated a rapid distribution of ^{11}C-MPGA in several organs. The highest activity was detected in the intestines, liver and kidneys. Brain activity was low throughout compared to these organs. The human whole-body study yielded similar results, with the intestines, liver and kidneys showing the highest activity. The estimated dose to the urinary bladder compartment turned out to be significant. The mean effective dose was 4.8 μSv/MBq (s.d. = 0.5 μSv/MBq). **Conclusion:** PET studies using 185 MBq (5 mCi) ^{11}C-MPGA are within the International Commission on Radiological Protection risk Category II for healthy volunteers.

Key Words: carbon-11-methoxyprogabidic acid; dosimetry; PET
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Gamma-amino-butyric acid (GABA) is the most important inhibitory neurotransmitter of the brain. It is estimated that 20%–50% of neurons, particularly those involved in local interneuronal circuits in the cerebral cortex, use GABA as a neurotransmitter (*1*). The GABA system is implicated in many neurological disorders, such as epilepsy and movement disorders. Different types of receptors have been identified. The $GABA_A$ receptor is a polypeptide linked to a chloride influx channel and is composed of five subunits. The subunit composition is important in terms of affinity for different ligands such as benzodiazepines, barbiturates and GABA itself, which each bind to specific sites in this receptor (*2–4*). The knowledge of $GABA_B$ receptors is less extensive. They seem to be coupled to G-proteins and might be located presynaptically on glutamatergic terminals (*5*). In vivo evaluation of GABA receptors using PET is possible using labeled ligands for these receptors. The most widely available of these tracers is ^{11}C-flumazenil, which is a ligand for the benzodiazepine binding site of $GABA_A$ receptors. These sites are different from the genuine GABA binding sites. Carbon-11-flumazenil does not bind to $GABA_B$ receptors.

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For correspondence or reprints contact: Patrick Santens, MD, Department of Neurology, University Hospital of Gent, De Pintelaan 185, 9000 Gent, Belgium.

Carbon-11-methoxyprogabidic acid (^{11}C-MPGA) (4-[{(4-[^{11}C] methoxyphenyl)(5-fluoro-2-hydroxyphenyl)methylene}amino]butyric acid) is a synthetic derivative of progabide (PGB) (4-[{(4-chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene}amino]butyramide) and its acid metabolite progabidic acid (PGA) (4-[{(4-chlorophenyl) (5-fluoro-2-hydroxyphenyl)methylene}amino]butyric acid).

Progabide is a synthetic GABA-prodrug. In the brain, PGB is rapidly converted to PGA and then more slowly to GABA (*6*). Progabidic acid has a GABA-mimetic activity at both $GABA_A$ and $GABA_B$ receptors, but its affinity is still inferior to that of GABA (*7*). The GABA-mimetic activity of PGB comes down to receptor binding by its metabolite PGA (*6–8*). There is convincing evidence that both compounds bind at GABA recognition sites in the respective receptor populations (*7,9*).

The synthesis of labeled PGA (^{11}C-MPGA) as a potential tracer for PET studies of the GABA-receptor has recently been described by our group (*10*). Toxicity of unlabeled PGB and PGA has been studied in the course of commercializing PGB as an antiepileptic drug (Gabrène®, Synthélabo, France). Single-dose administration in animals and humans was well tolerated, although in repeated and prolonged administration there is risk of hepatotoxicity (*11*).

We describe the biodistribution of ^{11}C-MPGA in animals and in humans. Based on these data, absorbed dose estimates are calculated for humans. Knowledge of the radiation absorbed dose is particularly relevant because of the increasing use of radioligands in diagnostic procedures in nuclear neurology.

MATERIALS AND METHODS

Synthesis

Carbon-11-MPGA was synthetized by oxygen-methylation of (4-hydroxyphenyl)-(5-fluoro-2-hydroxyphenyl)-methylene with $^{11}CH_3I$ and a subsequent Schiff's reaction with GABA (*10*). Purification was done by RP-HPLC and filtration through a 0.22 μm-pore membrane filter. An injectable solution with a specific activity of 11.1 GBq/μmol (300 mCi/μmol) was obtained at the end of synthesis and purification. The total activity obtained was 2.59 GBq (70 mCi). Chemical and radiochemical purity were found to be ≥97%.

Animal Studies

Eighteen male mice, of the NMRI strain, were given free access to food and water up to the time of the experiment. The mice were injected intravenously in the tail with 3.7 MBq (100 μCi) ^{11}C-MPGA dissolved in 200 μl isotonic phosphate buffer. At each of six preset time intervals postinjection (5, 10, 20, 40, 60 and 120 min), three animals were killed. The blood was collected and the organs were washed in a 0.9% NaCl solution. The weight of the organs was then determined. The activity was measured by a one-channel γ-spectrometer equipped with a 2 × 2 in NaI well

TABLE 1
Activity Detected in Mouse Organs After Intravenous Injection*

Time (min)†	Blood	Heart	Liver	Intestines	Kidneys	Brain	Remainder
5	6.13 (1.26)	17.73 (9.13)	14.32 (2.35)	2.73 (0.87)	21.45 (8.38)	1.77 (0.82)	1.93 (0.68)
10	4.58 (0.71)	9.12 (2.70)	16.04 (2.81)	7.00 (1.81)	16.38 (9.24)	4.24 (1.59)	1.47 (0.73)
20	3.65 (1.13)	4.83 (3.27)	12.32 (2.16)	11.74 (2.97)	9.40 (1.24)	5.26 (2.39)	1.51 (0.60)
30	1.23 (0.55)	2.93 (1.28)	8.67 (2.90)	18.68 (3.03)	5.13 (2.32)	2.10 (0.33)	0.88 (0.55)
60	1.33 (0.55)	2.02 (1.11)	8.39 (3.87)	17.42 (2.51)	5.22 (2.17)	1.56 (0.60)	0.95 (0.48)
120	0.45 (0.07)	0.23 (0.29)	3.00 (0.65)	23.01 (2.97)	1.61 (0.34)	0.06 (0.03)	0.50 (0.26)

*Intravenous injection is 3.7 MBq ^{11}C-MPGA and values are expressed as % of injected activity per gram of organ tissue (s.d.).
†n = 3 for each time interval.

counter (Canberra, UK) and corrected for physical decay and geometry. Results were expressed as percent of injected activity per gram of organ tissue and as percent of injected activity per organ. Time-activity curves for various organs were generated by applying least-squares to the raw data. All animal experiments were performed according to ethical rules governing such work.

Human PET Studies

Total-body PET studies on human volunteers were performed using a Siemens ECAT-951/31 positron camera (Siemens, Knoxville, TN) which was cross-calibrated with a NaI well counter. PET studies were performed on three healthy male volunteers (mean age 39 yr, mean weight 85 kg), after an intravenous bolus injection of 37 MBq (1 mCi) ^{11}C-MPGA. Six consecutive positions covered the entire thoracic cavity, the abdomen and the pelvis. Data acquisition was started 5 min postinjection, and data acquisition time was 5 min per position. For each position, a transmission scan was performed before the injection of the tracer, and 31 decay- and attenuation-corrected slices were reconstructed using standard Siemens ECAT software. The different organs were manually delineated in each of the six positions. The total activity of each organ was then calculated.

At 1 hr after injection, urine was collected from two of the three volunteers to determine urinary excretion. The urinary activity was measured in an isotope calibrator and corrected for physical decay. There was informed consent from all participants, and the Medical Ethics Committee of University Hospital of Gent approved all human experiments.

Dosimetry

The MIRDOSE 3 protocol was used to calculate the radiation absorbed dose in the human volunteers (*12,13*). This requires estimating residence times in source organs, which normally necessitates repetitive measures of organ activity over time in humans. Because this is nearly impossible with short-lived isotopes such as ^{11}C ($T_{1/2}$ = 20 min), we have used an alternative approach. The measured human organ activity was implemented on the fitted animal time-activity curves. This yielded time-activity curves for human organs allowing calculation of residence times according to the following equation:

$$A_0 \times T_{res} = \text{cumulated activity},$$

where A_0 is the injected activity and T_{res} is the residence time. A dynamic bladder model assuming a 1-hr voiding interval and a 65% urinary excretion of activity, known from the literature (*14*), was included.

RESULTS

Animal Studies

The distribution of ^{11}C activity in mice in various organs, expressed as a percent of injected activity per gram, of organ tissue at preset time points, is shown in Table 1. Figure 1 demonstrates the fitted time-activity curves of different organs expressed as a percent of injected activity. From these results, it is clear that the tracer is widely and rapidly distributed in various organs. Ten minutes after injection, more than 95% of the injected dose already was detected in the extravascular compartment. This was reflected in a rapid decline in blood and kidney activity. Activity in the liver decreased more slowly. At 2 hr postinjection, the total activity in the liver had dropped to 5% of injected activity. The activity in the intestines, on the contrary, rose steadily up to 84% of injected activity at 120 min postinjection.

Biodistribution in Humans

Total-body PET scan images of the human volunteers showed the highest activity in the liver, intestines, kidneys and urinary bladder. Heart activity was ascribed to passive accumulation in the blood in the early phases after injection.

Urinary bladder content was particularly relevant, as it had not been possible to evaluate this in the animal studies. For two of the three volunteers, urine was collected 60 min postinjection. It appeared that 3.55 and 3.88 MBq (96 and 105 μCi), respectively, were excreted in the urine. This was approximately 10% of the injected activity, which constituted a considerable input in the bladder content compartment. A large fraction of the injected activity was not detected in the organs mentioned. This fraction was considered the remainder of the body.

Human Dosimetry

On the basis of these data obtained in animal experiments and total-body human PET data, small intestine, kidneys, liver, urinary bladder content and the remainder of the body were considered the source organs for input in MIRDOSE 3. The small intestine activity also accounted for the stomach, large intestine, pancreas and spleen activity, as these organs could not be separately delineated.

The effective dose was estimated from MIRDOSE 3 as was defined by the International Commission on Radiological Protection (*15*). The values for the three volunteers and the estimated dose to the relevant organs are shown in Table 2. The mean estimated dose for the three volunteers was 4.8×-3 mSv/MBq (1.8×-2 rem/mCi). The standard deviation was 0.5×-3 mSv/MBq (0.2×-2 rem/mCi).

DISCUSSION

Neuronal activity in the brain is regulated by both excitatory and inhibitory neurotransmitters. Gamma-amino-butyric acid is the most important of these inhibitory neurotransmitters and, therefore, in vivo assessment of the GABA system might have implications for our knowledge of diseases such as epilepsy.

Progabidic acid has a proven GABA-mimetic activity at GABA binding sites, irrespective of the receptor subtype (*7*),

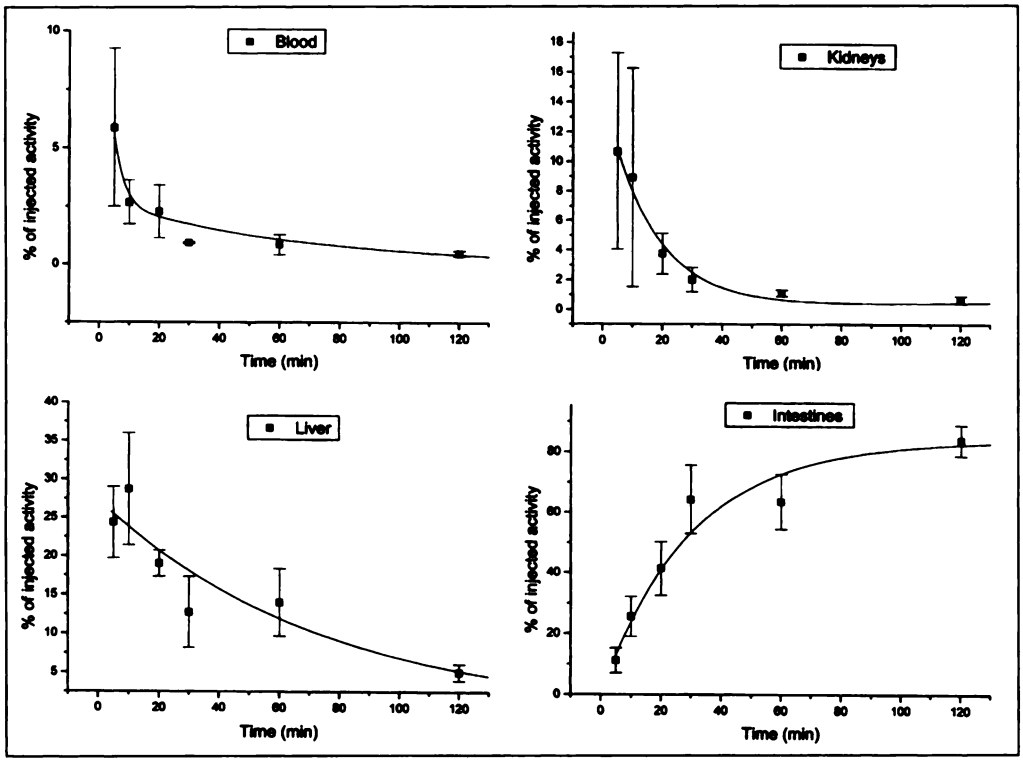

FIGURE 1. Fitted time-activity curves of organs after intravenous injection with 3.7 MBq ^{11}C-MPGA in male mice of the NMRI strain. Values are expressed as percent of injected activity with standard deviation indicated by error bars (n = 3 for each time interval).

unlike benzodiazepine receptor-binding substances, which bind only to $GABA_A$ receptors. This is the rationale for considering labeled analogs of PGA, such as ^{11}C-MPGA, for PET studies of the GABA receptor system in the human brain.

Biodistribution and dosimetry should be studied for any new tracer before using it for routine or research purposes. This is especially the case if the intended patient population is subjected to several other diagnostic nuclear medicine procedures.

We have studied the biodistribution of ^{11}C-MPGA in mice and humans. These results were similar in that the intestines, liver and kidneys bore the highest activity in both species. We found that in humans approximately 10% of the injected activity was excreted in urine at 1 hr postinjection, which necessitates consideration of an important bladder compartment. It was clear from the literature that after oral administration of labeled PGB in humans most activity is excreted in the urine (65%) (*14*). There is no information in the literature of excretory pathways in humans after intravenous administration of PGB or PGA. Excretion of activity in mice after intravenous injection of labeled PGB was almost equal in urine and feces (*14*).

Biliary excretion alone probably is not sufficient to explain the relatively early accumulation of activity in the intestines. Binding to aspecific or even specific receptor sites might be a reasonable alternative explanation for this finding.

Low activity was detected in the brain, which was of primary interest to us. This was probably the consequence of the acidic nature of ^{11}C-MPGA resulting in difficult passage through the blood-brain barrier.

The pharmacokinetic differences between species that we have mentioned might have implications for our strategy of implementing human data on animal time-activity curves for calculating residence times for human organs. We have used this approach as it is nearly impossible to gather total-body data in humans at different points in time with a short-lived isotope such as ^{11}C ($T_{1/2}$ = 20 min).

An alternative approach would consist of assuming instantaneous deposition of activity in the organs and the neglect of biological clearance. In this approach, the radiation dose to organs would be determined exclusively by radioactive decay. This assumption is supported by the reported biological half-life of unlabeled PGA, which ranges from 4–10 hr in different studies, (*11,16*) which is long, compared to the physical half-life of ^{11}C. This crude and conservative approach would result in a mean estimated dose for the three human volunteers of 5.3×-3 mSv/MBq (1.9×-2 rem/mCi). This value differs only slightly from the 4.8×-3 mSv/MBq (1.8×-2 rem/mCi) obtained using the animal data.

CONCLUSION

There is a rationale for considering ^{11}C-MPGA as a potential tracer for GABA receptors in the human brain. Using biodis-

TABLE 2
Radiation Dose to the Critical Organs Estimated by MIRDOSE 3*

	Dose Volunteer 1	Dose Volunteer 2	Dose Volunteer 3	Mean values	s.d.
Small intestine	2.0E-2 (7.5E-2)	4.8E-2 (1.8E-1)	3.1E-2 (1.2E-1)	3.3E-2 (1.3E-1)	1.4E-2 (5.3E-2)
Bladder wall	1.4E-2 (5.0E-2)	1.3E-2 (5.0E-2)	1.3E-2 (4.9E-2)	1.3E-2 (5.0E-2)	5.8E-4 (5.8E-4)
Kidneys	3.4E-3 (1.2E-2)	2.2E-2 (8.1E-2)	1.8E-2 (6.7E-2)	1.5E-2 (5.3E-2)	9.8E-3 (3.7E-2)
Liver	3.2E-3 (1.2E-2)	2.9E-3 (1.1E-2)	1.6E-2 (6.0E-2)	7.4E-3 (2.8E-2)	7.5E-3 (2.8E-2)
Effective dose	4.2E-3 (1.5E-2)	5.2E-3 (1.9E-2)	5.0E-3 (1.8E-2)	4.8E-3 (1.8E-2)	5.3E-4 (2.1E-3)

*Radiation dose to organs is mGy/MBq (rad/mCi), and effective dose values are mSv/MBq (rem/mCi).

tribution data in mice and humans, we demonstrated that the absorbed radiation dose is low, and administration of 185 MBq (5 mCi) ^{11}C-MPGA is sufficiently safe to remain in the effective dose range of 0.1–10 mSv, which corresponds to risk Category II as defined by the International Commission on Radiological Protection (*17*).

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