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Radiosynthesis and in vivo evaluation of [^{11}C]MC80 for P-glycoprotein imaging

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ABSTRACT

P-glycoprotein (P-gp) is an ATP-dependent efflux pump protecting the body against xenobiotics. The in vitro characterized modulator 6,7-dimethoxy-2-(6-methoxy-naphthalen-2-ylmethyl)-1,2,3,4-tetrahydroisoquinoline (MC80) of the P-gp pump was labelled with ^{11}C and evaluated in vivo for its potential to image P-gp function and expression. Radiochemical pure (>98%) [^{11}C]MC80 was obtained within 25 min starting from [^{11}C]methyl iodide with radiochemical yield of 26%. Biodistribution studies in FVB mice demonstrated a high baseline brain uptake (7.66 ± 1.38%ID/g at 1 min pi). Cerebral uptake was increased in mdr1a knock-out mice as well as after CsA pretreatment. Pre-administration of an excess of non-radioactive MC80 caused a reduced uptake in several target organs including brain, pancreas and intestines. The results indicate that [^{11}C]MC80 kinetics are modulated by P-gp. Reversesd phase-HPLC analysis of brain revealed an excellent metabolic profile (>90% intact [^{11}C]MC80).

1. Introduction

P-glycoprotein (P-gp) is an ATP-dependent efflux pump that is expressed in several normal tissues including liver, kidneys, intestine and brain.[1,2] The function of this efflux pump is to protect the human body against xenobiotics. It prevents accumulation in the brain of a wide range of drugs including anti-epileptics, anti-HIV drugs and antidepressants.[3–7] Overexpression of P-gp in tumours is a major reason of resistance to chemotherapeutics in some cancers.[8,9] Besides its role in multidrug resistance, changes or abnormalities in P-gp expression and function are involved in the etiology and pathogenesis of several neurological diseases.[10–13] A decreased P-gp function, for example, diminishes the clearance of amyloid plaques, increasing the vulnerability to Alzheimer disease.[14,15]

Imaging of P-gp function and expression with positron emission tomography (PET) or single photon emission tomography (SPECT) could be of great importance in drug development and medicine. Non-invasive monitoring of P-gp could be applied to elucidate the role of P-gp in several human diseases and to evaluate the efficacy of new P-gp modulators. Several tracers have already been evaluated for P-gp modulation among them [^{11}C]verapamil,[16,17] [^{11}C]*N*-desmethyl-loperamide,[18–20] and [^{99m}Tc]sestamibi.[21,22] All these radiotracers have at least one limitation, including significant contamination with radiometabolites. Moreover, the reported radiotracers for P-gp are all aimed to visualize P-gp function but not the expression and quantification of P-gp. Their very low baseline brain uptake make it difficult to see minor changes in P-gp expression and are therefore not useful as imaging agent for the P-gp expression.

In the search for a superior radiotracer to image P-gp function and expression, we evaluated [^{11}C]-6,7-dimethoxy-2-(6-methoxy-naphthalen-2-ylmethyl)-1,2,3,4-tetrahydroisoquinoline ([^{11}C]MC-80) for imaging P-gp function and expression. Recently, Colabufo et al. developed several 6,7-dimethoxytetrahydroiso-quinoline derivatives as novel P-gp modulators. Their P-gp interacting mechanism and their potency towards P-gp was evaluated in vitro by combining three biological assays: [^{3}H]vinblastine transport inhibition, Apparent permeability (P_{app}) determination and ATPase activation. From these assays, MC80 (Fig. 1) can be hypothesized as a transported substrate with an interaction profile similar to cyclosporine A (CsA) and an enhanced potency compared to CsA.[23]

This study describes the synthesis and purification of [^{11}C]MC80. Specific activity, radiochemical purity and log $D_{7.4}$ are reported. To investigate the P-gp modulation characteristics, [^{11}C]MC80 is evaluated in wild-type mice with or without CsA

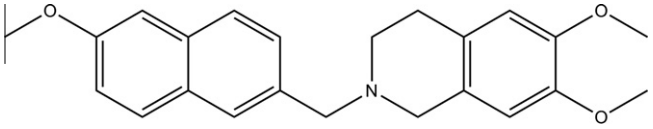

Figure 1. Chemical structure of MC80.

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pretreatment in addition to P-gp knock-out mice. The metabolic profile of [^{11}C]MC80 in all three treatment groups is determined. Finally, specific binding of [^{11}C]MC80 is examined by pre-administration of non-radioactive MC80.

2. Results and discussion

2.1. Chemistry

Compound **2** was synthesized by condensing the appropriate 2-naphtyl carboxylic acid with the isoquinoline moiety in the presence of carbonyldiimidazole. The precursor 6-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-ylmethyl)-naphtalen-2-ol (MC90) was prepared from **2** by reducing the amide with $LiAlH_4$ (Scheme 1). MC80 was prepared as previously decribed.[23]

2.2. Radiochemistry

The radiosynthesis of [^{11}C]MC80 is shown in Figure 2. The hydroxyl precursor was labelled with [^{11}C]methyl iodide ([^{11}C]-CH_3I) in the presence of sodium hydride as a base. [^{11}C]MC80 eluted with a retention time of 9.3 min and is adequate separated from MC90 (t_R = 5.5 min). [^{11}C]MC80 was prepared with an overall decay-corrected yield of 26 ± 5% (n = 6). Extraction efficiency of the Sep-pak was calculated to be 87 ± 3% (n = 6). The total synthesis, from the end of [^{11}C]CH_3I delivery to reaction vessel to delivery for in vivo studies was completed in 25 min.

2.3. Quality control and log $D_{7.4}$

[^{11}C]MC80 was readily prepared (radiochemical yield of 25%) for intravenous injection by O-methylation of MC90 with [^{11}C]methyl iodide under basic conditions. Purification by reversed phase-HPLC and Sep-pak provided [^{11}C]MC80 in high radiochemical purity (>98%) and high specific activity (>0.3 TBq/μmol). The identity of [^{11}C]MC80 (t_R = 11.1 min) was confirmed by co-elution with authentic reference compound, MC80 (t_R = 10.8 min), after co-injection on HPLC. The [^{11}C]MC80 formulation was chemically pure and remained stable during the time span of the experiments.

We determined log $D_{7.4}$ as an indicator for lipophilicity and blood–brain permeability according to the shake flask method. The log $D_{7.4}$ of [^{11}C]MC80 was 1.96 ± 0.08 using *n*-octanol as organic phase and Dubelcco's phosphate buffered saline (DPBS) as aqueous phase. This value is suitable for brain penetration.

2.4. Biodistribution studies

In vitro, MC80 was characterized as a transported substrate with an interaction profile similar to CsA.[23] The in vivo behaviour was assessed in wild-type mice as well as in mice lacking the P-gp encoding gene mdr1a. In addition, the influence of CsA and MC80 on the tissue distribution in wild-type mice was determined.

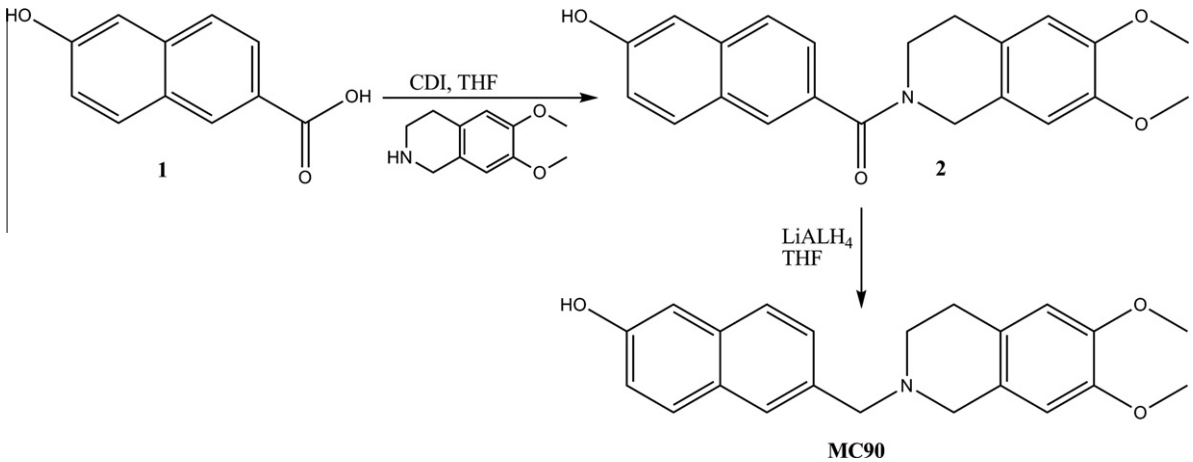
2.4.1. Biodistribution study in wild-type mice

The tissue distribution of [^{11}C]MC80 in wild-type mice with saline pretreatment is depicted in Figure 3. [^{11}C]MC80 displayed good uptake in mouse brain (7.66 ± 1.38%ID/g at 1 min pi) followed by efficient wash-out (0.90 ± 0.20%ID/g at 90 min pi). Blood activity never exceeded brain uptake. [^{11}C]MC80 is cleared from plasma via the hepatobiliary system as shown by the high liver uptake (8.71 ± 2.44%ID/g) and the increase in radioactivity uptake over time in small intestine (from 3.89 ± 0.30%ID/g at 1 min pi to 11.78 ± 3.38%ID/g at 90 min pi) and large intestine (from 1.83 ± 0.72%ID/g at 1 min pi to 2.18 ± 0.82%ID/g at 90 min pi). [^{11}C]MC80 also demonstrated high initial kidney uptake (11.32 ± 0.95%ID/g at 1 min pi), and subsequently urinary clearance was observed (data not shown). Since radioactivity uptake in the intestines already occurred at the earliest time point, it is probably not only caused by excretion of [^{11}C]MC80 but is possibly also P-gp mediated.

Compared to [^{11}C]verapamil[16,17] and [^{11}C]*N*-desmethyl-loperamide,[18–20] [^{11}C]MC80 displayed a high baseline brain uptake. This could be advantage for imaging small fluctuations not only in P-gp expression but also in P-gp function.

2.4.2. Biodistribution study in wild-type mice after CsA pretreatment

Pretreatment of animals with CsA caused a significant increase (except at 90 min pi) of radioactivity uptake in mice brain compared to FVB mice treated with physiological saline (Fig. 3). The calculated *p*-values are 0.003, 0.007, 0.011, 0.009 and 0.293 at 1, 10, 30, 60 and 90 min pi, respectively. Brain uptake raised 1.3–2-fold compared to mice without CsA pretreatment. Except at 1 min pi, CsA also increased the uptake of [^{11}C]MC80-derived radioactivity in several peripheral organs including testes (from 2.56 ± 0.51%ID/g to 3.73 ± 0.66%ID/g at 60 min pi), pancreas (from 6.85 ± 0.66%ID/g to 8.19 ± 0.79%ID/g at 60 min pi), spleen (from 1.52 ± 1.12%ID/g to 2.31 ± 0.15%ID/g at 60 min pi), kidneys (from 2.46 ± 1.61%ID/g to 4.53 ± 0.28%ID/g at 60 min pi), and liver (from 4.86 ± 0.33%ID/g to 8.25 ± 0.99%ID/g at 60 min pi). This increase however was not significant at every time point. The increase in testes could be expected



Scheme 1. Synthesis of MC90.

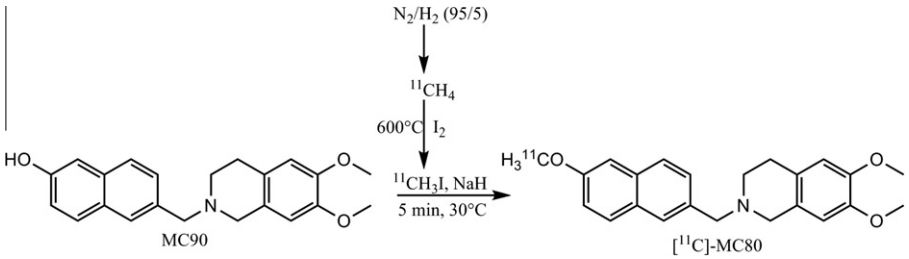


Figure 2. Radiosynthesis of [^{11}C]MC80.

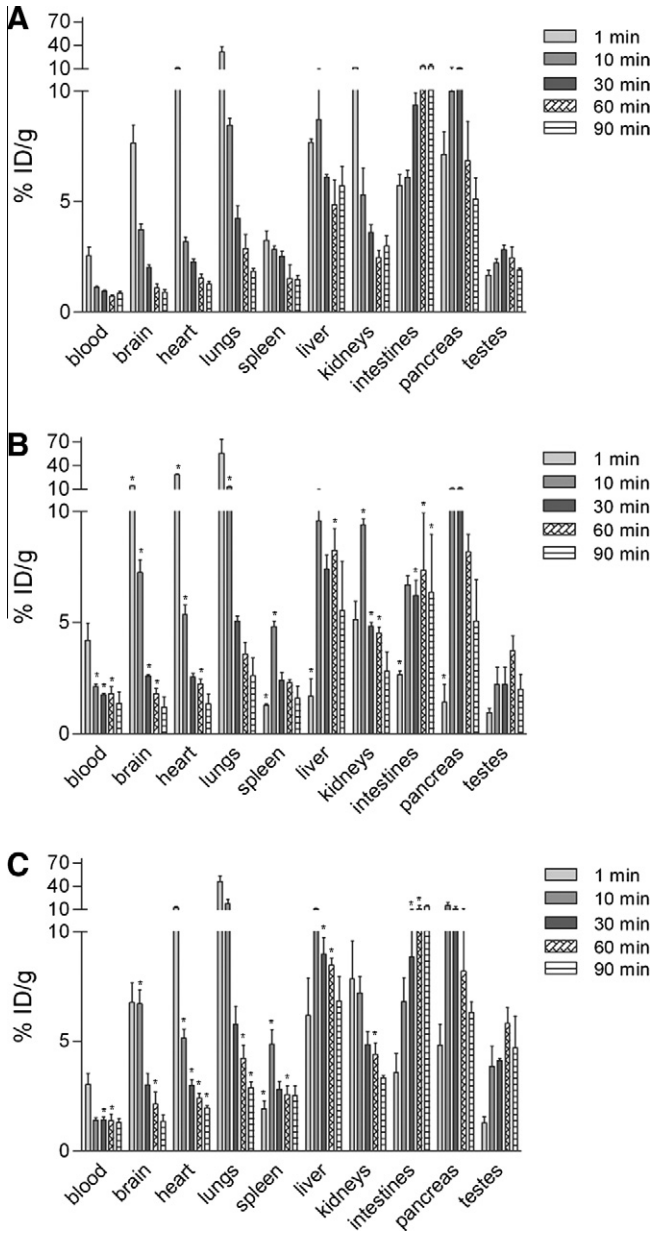


Figure 3. Uptake (%ID/g) of radioactivity in selected mice organs at various time points after iv injection of 4.5 MBq (122 μCi) [^{11}C]MC80. (A) Pretreatment with saline in FVB mice (n = 3), (B) pretreatment with CsA (50 mg/kg) in FVB mice (n = 3) and (C) pretreatment with saline in mdr1a (−/−) mice (n = 3).

since P-gp is also localized at the testes. The testes/blood ratio however is decreased (Table 1). This can be explained by the increase in blood radioactivity levels in mice pretreated with CsA. Compared to mice treated with saline, radioactivity uptake in blood (from 2.56 ± 0.67%ID/g to 4.97 ± 0.26%ID/g), heart (from 10.69 ± 2.49%ID/g to 28.55 ± 1.35%ID/g) and lungs (from 31.35 ± 11.60%ID/g to 71.59 ± 15.79%ID/g) was already higher at 1 min pi and remained higher at each time point. These observations suggest a slower distribution of [^{11}C]MC80 to the peripheral organs after CsA administration. In contrast, intestinal uptake was significantly lower with CsA pretreatment. A possible reason is the reduced interaction with P-gp caused by CsA modulation. Taken together, CsA administration has an effect on tissue uptake of [^{11}C]MC80 suggesting P-gp plays a role in the kinetics of [^{11}C]MC80. However, it is not likely that [^{11}C]MC80 is a P-gp substrate with high-affinity since brain/blood ratio did not show a significant increase compared to the mice without CsA pretreatment. Moreover, the increase in brain uptake seem to be mainly driven by changes in blood activity concentrations rather then by enhanced uptake in the brain (Table 1). This is in accordance with the in vitro data that showed that MC80 did not stimulate ATPase activity and it is known that stimulation of ATPase activity is a good marker for identifying a high-affinity substrate.

2.4.3. Biodistribution study in mdr1a knock-out mice

In mdr1a (−/−) mice, cerebral uptake of [^{11}C]MC80 was significant increased at 10 min (p = 0.014) and 60 min pi (p = 0.001) compared to wild-type mice (Fig. 3). The highest increase in brain uptake was 1.96 ± 0.38 (from 1.09 ± 0.18%ID/g to 2.15 ± 0.65%ID/g) at 60 min pi. At 1 min pi most organs displayed a reduced radioactivity uptake. In contrast lungs (from 31.35 ± 11.60%ID/g to 45.85 ± 13.79%ID/g) and heart (from 10.69 ± 2.49%ID/g to 13.06 ± 1.72%ID/g) showed an increased uptake at 1 min pi. As with CsA, these observations suggest also a slower distribution of [^{11}C]MC80 to peripheral organs when using mdr1a (−/−) mice (Fig. 3). Genetic disruption of the mdr1a gene appears to affect retention of the tracer in the excretory organs. Radioactivity uptake in the kidney raised from 2.46 ± 1.61%ID/g to 4.41 ± 0.63%ID/g at 60 min pi, and liver uptake increased from 4.86 ± 0.33%ID/g to 8.47 ± 0.57%ID/g at 60 min pi. The elevation in testes (significant at 10 min (p = 0.015) and 30 min (p = 0.003) pi) uptake is in accordance with P-gp distribution. In addition to the testes radioactivity uptake, the testes-to-blood ratio is also increased in mdr1a (−/−) mice (Table 1). The highest raise in the testes-to-blood ratio is observed at 60 min pi (1.8-fold increase). However the elevation was not significant at any of the investigated time points. Contrary to the effect seen with CsA pretreatment, intestinal uptake did not alter. This can be explained by the fact that mdr1b encoded P-gp is also found in the gastrointestinal tract resulting in an unchanged intestinal uptake when using mdr1a (−/−) mice.

CsA administration, as well as genetic disruption of the mdr1a gene, resulted in significant changes in tissue distribution, confirming that [^{11}C]MC80kinetics are modulated by P-gp not only in vitro but also in vivo. However, the increase in brain radioactivity is not as high as for [^{11}C]*N*-desmethyl-loperamide and [^{11}C]verapamil indicating that although [^{11}C]MC80 is modulated by P-gp it is not a good substrate for P-gp. Therefore, it is likely that the increased

Table 1
Brain-to-blood and testes-to-blood ratios of radioactivity uptake of [^{11}C]MC80

Group	Tissue	1 min	10 min	30 min	60 min	90 min
Control[a]	Brain/blood	3.07 ± 0.58	3.38 ± 0.66	2.17 ± 0.17	1.51 ± 0.15	1.05 ± 0.11
	Testes/blood	0.68 ± 0.23	2.02 ± 0.40	3.04 ± 0.40	3.38 ± 0.56	2.27 ± 0.27
CsA treatment[b]	Brain/blood	2.91 ± 0.02	3.40 ± 0.13	1.50 ± 0.17	1.04 ± 0.29	0.93 ± 0.24
	Testes/blood	0.23 ± 0.05	1.02 ± 0.56	1.26 ± 0.77	1.33 ± 0.97	1.53 ± 0.22
mdr1a (−/−) mice[c]	Brain/blood	2.34 ± 0.67	4.91 ± 1.57	2.10 ± 0.37	1.09 ± 0.97	1.02 ± 0.15
	Testes/blood	0.51 ± 0.35	2.51 ± 0.66	2.67 ± 0.21	4.43 ± 1.49	4.13 ± 1.61

Mice were iv injected with 18.5 MBq (500 μCi) [^{11}C]MC80.
[a] Pretreatment with saline in FVB mice (n = 3).
[b] Pretreatment with CsA (50 mg/kg) in FVB mice (n = 3).
[c] Pretreatment with saline in mdr1a (−/−) mice (n = 3).

brain uptake is due to a higher delivery to the brain and not to a reduction in wash-out.

2.4.4. Biodistribution study after pretreatment with cold compound

The tissue distribution of [^{11}C]MC80 in wild-type mice with and without cold MC80 pretreatment is depicted in Table 2. The brain uptake of [^{11}C]MC80 was reduced by 20% after pretreatment of the animals with non-radioactive MC80. This reduced uptake was significant at 30 min pi (p = 0.037). In the pancreas, pretreatment caused the most outstanding decline in tracer uptake (reduction with 52% and 49% at 30 min and 60 min pi, respectively). A significant decreased uptake of [^{11}C]MC80 was also noticed in the heart (reduction with 22% and 21% at 30 min and 60 min pi), lungs (reduced with 47% at 60 min pi), small intestines (reduction with 41% and 44% at 30 min and 60 min pi, respectively), large intestines (reduction with 26% and 35% at 30 min and 60 min pi, respectively) and testes (reduced with 24% at 30 min pi).

Summarizing, the highest amount of reduction of [^{11}C]MC80 radioactivity occurred in the pancreas, lungs and intestine. A lower fraction of radioactivity decrease occurred in the brain, testes and heart. The organs that displayed reduced [^{11}C]MC80 levels upon administration of non-radioactive MC80 reflects the known expression pattern of P-gp.[26,27]

2.5. Metabolite analysis

The stability of [^{11}C]MC80 in vivo was studied at 10 and 30 min pi. HPLC measurements of plasma and brain spiked with [^{11}C]MC80, displayed one radioactive peak (retention time = 10 min). This peak corresponds with [^{11}C]MC80 as it was found to co-elute with authentic MC80.

The metabolic profile of [^{11}C]MC80 in the three different treatment regiments is summarized in Table 3.

2.5.1. Metabolism study in wild-type mice

After protein elimination, the recovery of radioactivity in the supernatant solutions was 97 ± 1% in plasma and 87 ± 7% in brain. In brain, [^{11}C]MC80 remained very stable with over 98% and 91% intact product at 10 and 30 min pi, respectively. The tracer underwent a more extensive metabolization in plasma. Plasma metabolite analysis showed 63 ± 6% and 58 ± 6% intact [^{11}C]MC80 at 10 and 30 min, respectively. Only one polar metabolite was found, that eluted at the void volume of the column. When [^{11}C]MC80 is demethylated, it is anticipated that ^{11}C-methyl is removed and metabolized to $^{11}CH_3OH$, $^{11}CH_2O$, $^{11}CO_2H$ or $^{11}CO_2$. These compounds are very polar and are believed to correspond with the radioactive peak of the solvent front.

2.5.2. Effect of CsA pretreatment on the in vivo stability of [^{11}C]MC80

The radioactivity recovery in supernatant was not changed compared with the treatment group without CsA administration. In both tissues, the same radioactive fractions were found as without CsA pretreatment (Table 3). Administration of CsA did not affect the metabolic profile of [^{11}C]MC80 in brain. The percentage intact [^{11}C]MC80 was 99% and 93% at 10 and 30 min, respectively. Those values are similar to mice pretreated with saline. Blood metabolite studies indicated that [^{11}C]MC80 underwent an

Table 2
Tissue distribution of [^{11}C]MC80 in FVB mice with (Test group) and without (Control group) administration of non-radioactive MC80 (15 mg/kg)

	%ID/g ± SD (n = 3)			
	Control group		Test group	
	30 min	60 min	30 min	60 min
Blood	0.93 ± 0.09	0.73 ± 0.05	0.82 ± 0.06	0.78 ± 0.13
Brain	2.03 ± 0.20	1.09 ± 0.18	1.64 ± 0.28*	0.89 ± 0.16
Heart	2.26 ± 0.24	1.54 ± 0.19	1.75 ± 0.25*	1.21 ± 0.16*
Lungs	4.24 ± 0.98	2.86 ± 0.56	3.08 ± 0.33	1.70 ± 0.11*
Stomach	7.72 ± 6.74	3.37 ± 0.62	2.21 ± 1.17	2.46 ± 0.71
Spleen	2.53 ± 0.38	1.52 ± 1.12	1.80 ± 0.70	1.31 ± 0.13
Liver	6.10 ± 0.20	4.86 ± 0.33	5.39 ± 0.80	5.63 ± 0.26
Kidneys	3.62 ± 0.59	2.46 ± 1.61	2.99 ± 0.93	2.10 ± 0.31
Small intestine	8.03 ± 0.91	11.68 ± 0.13	4.77 ± 0.88*	6.54 ± 1.62*
Large intestine	1.33 ± 0.07	1.45 ± 0.78	0.98 ± 0.02*	0.95 ± 0.10*
Bladder	4.45 ± 1.16	4.00 ± 1.77	3.86 ± 0.60	4.71 ± 1.34
Pancreas	10.63 ± 1.68	6.85 ± 0.66	5.15 ± 0.30*	3.53 ± 0.37*
Testes	2.83 ± 0.36	2.46 ± 0.51	2.15 ± 0.12*	2.10 ± 0.28

[^{11}C]MC80; Values are averaged and decay-corrected.
* p <0.05 using one-sided, unpaired student's t-test; Mice were iv injected with 4.5 MBq (122 μCi).

Table 3
Metabolic profile of [^{11}C]MC80

Treatment group	Tissue	Time (min)	Retention time on RP-HPLC	
			2.5 min	10 min [^{11}C]MC80
Control[a]	Brain	10	2 ± 2%	98 ± 2%
		30	9 ± 1%	91 ± 1%
	Plasma	10	37 ± 6%	63 ± 6%
		30	42 ± 6%	58 ± 6%
CsA pretreatment[b]	Brain	10	1%	99%
		30	7 ± 1%	93 ± 1%
	Plasma	10	35 ± 9%	65 ± 9%
		30	78 ± 3%	22 ± 3%
mdr1a(−/−) mice[c]	Brain	10	2 ± 1%	98 ± 1%
		30	8 ± 2%	92 ± 2%
	Plasma	10	23 ± 7%	77 ± 7%
		30	40 ± 3%	60 ± 3%

Mice were iv injected with 18.5 MBq (500 μCi) [^{11}C]MC80; Results (averaged and decay-corrected) are expressed as percentages of the total activity ± SD.
[a] Pretreatment with saline in FVB mice (n = 3).
[b] Pretreatment with CsA (50 mg/kg) in FVB mice (n = 3).
[c] Pretreatment with saline in mdr1a (−/−) mice (n = 3).

elevated metabolization when CsA was given; at 30 min pi, the percentage unchanged [^{11}C]MC80 was 58 ± 6% without and 22 ± 3% with CsA pretreatment.

2.5.3. Metabolism study in mdr1a knock-out mice

Similar to the previous reported treatment regiments, two radioactive products are detected (Table 3). The use of mdr1a (−/−) mice did not affect the amount of unchanged [^{11}C]MC80 present in brain. At 10 min pi, 98 ± 1% of parent compound remained in the brain and at 30 min pi 92 ± 2% was still present as unchanged [^{11}C]MC80. The fraction of radioactivity in plasma that represented [^{11}C]MC80 was 77 ± 7% at 10 min pi and 60 ± 3% at 30 min pi representing the same metabolic profile as in FVB mice.

3. Conclusions

[^{11}C]-MC80 was readily prepared (radiochemical yield of 25%) for intravenous injection by methylation of MC90 with [^{11}C]methyl iodide, itself prepared from cyclotron-produced [^{11}C]methane. Purification by RP-HPLC and Sep-pak provided [^{11}C]-MC80 in high radiochemical purity (>98%) and very high specific activity (>0.3 TBq/μmol).

Biodistribution studies in wild-type mice demonstrated brain uptake which quickly maximized and then washed out. Compared to [^{11}C]*N*-desmethyl-loperamide and the clinical used [^{11}C]verapamil, [^{11}C]MC80 has a high initial brain uptake which could be advantage for measuring small changes in P-gp expression. [^{11}C]MC80 kinetics are modulated by P-gp. Cerebral radioactivity uptake was only increased slightly in P-gp knock-out mice and after CsA pretreatment, pointing out that [^{11}C]MC80 is not a suitable tracer for imaging P-gp function a the BBB. However, intestinal [^{11}C]MC80 radioactivity uptake is highly sensitive towards P-gp indicating [^{11}C]MC80 has the potential for imaging intestinal P-gp expression.

HPLC measurements demonstrated that [^{11}C]MC80 has an outstanding metabolic profile compared to [^{11}C]verapamil and is slightly better than [^{11}C]*N*-desmethyl-loperamide.[16–20] At 30 min pi over 90% of the measured radioactivity in brain was related to unchanged [^{11}C]MC80. Structural analogues possessing the same metabolic stability in vivo, but with a higher affinity and specificity for P-gp may be extremely useful for imaging of P-gp expression at the BBB.

4. Experimental

4.1. General procedures and materials

All chemicals were purchased from commercial sources (Sigma-Aldrich or Acros Organics, Belgium) and were used without further purification. CsA (Sandimmune®) was purchased in a concentration of 250 mg/mL (Novartis Pharma, Vilvoorde, Belgium). Solvents were purchased from Lab-Scan Analytical Sciences (Dublin, Ireland).

Unless otherwise mentioned, the HPLC system used consisted of a Waters 515 HPLC pump, a Waters 2487 UV detector (Waters, Milford, USA) set at 254 nm, a Ludlum model 2200 scalar ratemeter equipped with a Geiger Müller tube (Ludlum Measurements Inc., Sweetwater, USA) and a Shimadzu C-RSA chromatopac data analyser. Absorption units full scale were set at 0.0001. The columns, mobile phases and flow rates are indicated in the text below.

All radioactivity counting was performed with a Cobra gamma-ray spectrometer equipped with five 1 × 1 inch NaI(Tl) crystals (Cobra autogamma, Packard Canberra). The values are corrected for background radiation and physical decay during counting.

All animal studies were conducted following the principles of laboratory animal care and the Belgian Law on the protection of animals. The performed experiments are approved by the local Ethical Committee of Ghent University. Wild-type mice (FVB strain) were purchased from Bioservices (Uden, The Netherlands). The mdr1a (−/−) mice, also referred to as P-gp knock-out mice, were obtained from Taconic (New York, USA). Mice were allowed free access to food and water.

Statistical analysis was performed using the unpaired, one-sided student's *t*-test. A $P < 0.05$ is considered as significant.

4.2. Chemistry

All reactions were conducted under N_2 atmosphere with dry solvents under anhydrous conditions. Column chromatography was performed with 1:30 Merck Silica Gel 60A (63–200 mesh) as the stationary phase using the solvent systems indicated in the text. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Elemental analyses (C, H and N) were performed on Eurovector Euro EA 3000 analyzer; the analytical results were within ± 0.4% of the theoretical values for the formula given. The ^{1}H NMR spectra were measured with a Varian 300 MHz Mercury-VX spectrometer. Chemical shift values are recorded in ppm (δ) from an internal tetramethylsilane standard in $CDCl_3$. Recording of mass spectra was done on an HP6890-5973MSD gas chromatograph/mass spectrometer; only significant *m*/*z* peaks, with their percentage of relative intensity in parentheses, are reported. All spectra were in accordance with the assigned structures.

4.2.1. (6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-(6-hydroxy-naphtalen-2-yl)-methanone (2)

A mixture of 6-hydroxy-2-naphthalenylcarboxylic acid (1 mmol) (**1**) and carbonyldiimidazole (1.1 mmol) in THF (50 mL) was stirred overnight at room temperature. 6,7-Dimethoxytetrahydroisoquinoline (1.0 mmol) dissolved in THF (20 mL) was added to the reaction mixture. After stirring for 8 h at room temperature, the reaction mixture was added to a separatory funnel containing H_2O (30 mL) and ethyl acetate (30 mL). The organic layer was washed with Na_2CO_3 (3 × 30 mL), dried on anhydrous Na_2SO_4 and evaporated. Purification by silica gel chromatography (CH_2Cl_2/EtAc 1:1) gave **2** as a white solid in a 32% yield. Mp 116–120 °C. Anal. ($C_{22}H_{21}NO_4 \cdot 0.8H_2O$) C, H, N. ESI$^+$/MS *m*/*z* 364 (ESI M$^+$+1, 100), 171 (M$^+$ 18). ^{1}H NMR: δ 2.87–2.92 (m, 3H, NCH_2CH_2 isoquinoline and OH, D_2O exchanged), 3.62–4.02 (m, 8H, 2H of CH_3 and NCH_2CH_2 isoquinoline 2H of CH_3), 4.62–4.87 (m, 2H, NCH_2 isoquinoline), 6.64–7.95 (m, 8H aromatic).

4.2.2. 6-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-ylmethyl)-naphtalen-2-ol (MC90)

Compound **2** (2 mmol) dissolved in THF (10 mL) was added drop-wise to a suspension of $LiAlH_4$ (2 mmol) in THF (30 mL) and the resulting mixture was refluxed for 2 h. Afterwards, the reaction mixture was cooled to room temperature and H_2O (1 mL) was added drop-wise to eliminate $LiALH_4$ excess. The reaction mixture was further diluted with H_2O (20 mL) and extracted with Et_2O (30 mL). The organic layer was separated and washed with 3 N HCl (3 × 10 mL). The combined aqueous fractions were alkalinized with solid Na_2CO_3 and extracted with $CHCl_3$ (3 × 20 mL). The organic layers were collected and dried on anhydrous Na_2SO_4. The solvent was removed under reduced pressure to afford a crude residue that was purified with column chromatography (CH_2Cl_2/AcEt 1:1) to yield MC90 (42%) as a white solid. Mp 207–209 °C. Anal. ($C_{22}H_{23}NO_3 \cdot 0.25H_2O$) C, H, N. ESI$^+$/MS *m*/*z* 350 (ESI M$^+$+1, 100), 194 (M$^+$ 68). ^{1}H NMR: δ 2.75–2.96 (m, 5H, NCH_2CH_2 isoquinoline and NCH_2 isoquinoline, OH, D_2O exchanged), 3.62 (s, 2H, NCH_2

naphthalene), 3.78–3.86 (m, 8H, NCH_2CH_2 isoquinoline 2H of CH_3), 7.00–7.70 (m, 8H aromatic).

4.2.3. 6,7-Dimethoxy-2-(6-methoxy-naphthalen-2-ylmethyl)-1,2,3,4-tetrahydro-isoquinoline (MC80)

Mp 232–236 °C. Anal. ($C_{23}H_{25}NO_3 \cdot HCl \cdot 0.5H_2O$) C, H, N. ESI^+/MS m/z 364 (ESI M^++1, 43), 171 (M^+ 100). ^1H NMR: δ 2.78–2.86 (m, 4H, NCH_2CH_2 isoquinoline and NCH_2 isoquinoline), 3.58 (s, 2H, NCH_2 naphthalene), 3.77–3.92 (m, 11H, 3H of CH_3 and NCH_2CH_2), 6.46–7.73 (m, 8H aromatic).

4.3. Radiochemistry

4.3.1. Production of [^{11}C]methyl iodide

The alkylating reagent [^{11}C]methyl iodide was prepared from [^{11}C]methane by gas-phase iodination. [^{11}C]CH_4 was produced in a Cyclone 18 twin cyclotron (IBA, Gent, Belgium) via the $^{14}N(p,\alpha)^{11}C$ reaction induced by irradiation of N_2 gas containing 5% H_2 with proton beam (18 MeV, 14 μA) for 20 min. At the end of the irradiation, [^{11}C]CH_4 was transferred from the cyclotron target to a homemade synthesis module housed in a hot cell. The transferred [^{11}C]CH_4 was trapped on a loop filled with Porapak N (divinylbenzene/vinyl pyrolidone polymer), cooled in liquid argon. Once all [^{11}C]CH_4 was released from the target, the loop was flushed to remove N_2/H_2 and allowed to warm to room temperature. The [^{11}C]CH_4 was swept of with a stream of helium and mixed with I_2 vapours at 50 °C. In a quartz tube heated to 600 °C, [^{11}C]CH_4 was converted to [^{11}C]CH_3I which was collected on a second Porapak N trap. The remaining iodine and produced HI were trapped in ascarite. The unreacted [^{11}C]CH_4 was further circulated until [^{11}C]CH_3I production reached a maximum. [^{11}C]CH_3I was subsequently released from the Porapak N trap by heating the trap to 120 °C.

4.3.2. Radiosynthesis of [^{11}C]MC80 with [^{11}C]MeI

[^{11}C]CH_3I in carrier helium was bubbled into a sealed vial containing MC90 (3 μmol) and sodium hydride (7 μL, 1 M in DMF) in DMF (243 μL). When the radioactivity in the reaction vial reached maximum activity levels, the reaction mixture was heated at 30 °C for 5 min. Afterwards, the crude reaction mixture was diluted with eluent and injected onto a semi-preparative C_{18} HPLC column (Econosphere, 10 μm, 10 × 250 mm, Grace Davison Discovery Sciences, Lokeren, Belgium) that was eluted at 6 mL/min with (80:20) MeOH/sodium acetate buffer (0.02 M, pH 5.5). The eluate was monitored for radioactivity (solar-blind P.I.N. photodiode) and UV absorbance (smartline UV detector 2500, Knauer, Berlin, Germany) at 254 nM. The isolated fraction containing [^{11}C]MC80 was diluted with DPBS (30 mL, pH 7.4, 0.01 M) and loaded on a C_{18} Sep-pak (Alltech Maxi-clean SPE Prevail C_{18}, previously activated with 1 mL EtOH and 5 mL sterile water). After the cartridge has been washed with sterile water (5 mL), the desired product [^{11}C]MC80 was eluted with EtOH (1 mL). For biodistribution studies, the EtOH fraction was diluted with physiological saline (10 mL). For metabolite analysis, EtOH was evaporated to dryness and the residue was redissolved in an adequate amount of EtOH/saline (8:92—v/v).

4.4. Radioanalytical data

4.4.1. Quality control

Determination of the radiochemical and chemical purity as well as the measurement of specific activity was performed by analytical HPLC assay using a Gracesmart C_{18} column (5 μm, 4.6 × 250 mm, Grace Davison Discovery Sciences, Lokeren, Belgium) at a flow rate of 1 mL/min. The mobile phase consisted of a mixture of 60% acetonitrile and 40% sodium acetate buffer (0.02 M, pH 5.5).

A calibration curve of unlabelled reference compound (0.02×10^{-3}–1×10^{-3} μM) was calculated and controlled for its accuracy and reproducibility. Additionally, the detection limit was determined. Specific activities were decay-corrected to the end of synthesis (EOS).

4.4.2. Reference control

An aliquot of [^{11}C]MC80 was co-injected with μmol unlabelled MC80 on the HPLC system to confirm its identity. Since the radioactive and the non-labelled reference co-eluted, their identity was confirmed.

4.5. Partition coefficient

The partition coefficient (expressed as log $D_{7.4}$) was measured according to the shake flask method.[24,25] Approximately 1.85–3.7 MBq (50–100 μCi) of [^{11}C]MC80 was added to a test tube containing DPBS (3 mL, pH 7.4, 0.01 M) and *n*-octanol (3 mL). The mixture was shaken vigorously by hand, vortexed for 3 min and centrifuged for 3 min at 3000 g. To remove hydrophilic impurities, the phosphate buffer was discarded and replaced by fresh one. Again the mixture was shaken vigorously by hand, vortexed for 3 min and centrifuged for 3 min at 3000*g*. An aliquot of 0.5 mL of both *n*-octanol phase and buffer phase was taken and counted separately for radioactivity in an automated gamma counter. The aqueous phase was discarded and fresh buffer (2.5 mL) was added. This process was repeated twice more. The partition coefficient was determined by calculating the ratio of radioactivity in the collected *n*-octanol fractions to the collected buffer solutions: (counts in *n*-octanol)/(counts in DPBS buffer). The measurements were done in triplicate.

4.6. Biodistribution studies

Wild-type mice or mdr1a knock-out mice of 5–7 weeks old weighing approximately 25 g were used. Mice (n = 3 for each time point of each treatment group) were injected in the tail vein with 150 μL EtOH/physiological saline (8:92) containing 4.5 MBq (122 μCi) [^{11}C]MC80. Thirty minutes before administration of [^{11}C]MC80, the mice were either treated with physiological saline or with 50 mg/kg CsA (250 mg/5 mL solution diluted with sterile water to a concentration of 10 mL/kg). At various periods after injection of the radioligand, the animals were sacrificed by cervical dislocation under isoflurane anaesthesia. Blood and urine were removed and organs were dissected. Tissues were weighed and counted for radioactivity using the gamma counter. The uptake of radioactivity in the tissues was expressed as percentage of the injected dose per gram of tissue plus or minus the standard deviation (%ID/g ± SD).

Specific binding of [^{11}C]MC80 was examined by pretreatment of FVB mice (n = 3 for each time point) with non-radioactive MC80 (15 mg/kg), 30 min before administration of the radioligand. At 30 and 60 min after [^{11}C]MC80 injection, animals were killed and dissected. Tissues were treated as described above. Results are decay-corrected and expressed as %ID/g ± SD. Statistical analysis was performed using one-sided, unpaired student's t-test. Only p-values <0.05 are considered significant.

4.7. Metabolite analysis

200 μL 92:8 (v/v)—physiological saline/EtOH containing approximately 18.5 MBq (500 μCi) [^{11}C]MC80 was injected in the tail vein of mice (5–7 weeks old, weighing 25–30 g). At 1, 10 and 30 min pi, the mice were sacrificed and blood and brain were taken. Blood was collected into a vacutest tube containing 3.6 mg K_3EDTA and was centrifuged at 4000 g for 6 min to

separate plasma. Plasma (200 μL) was mixed with 800 μL acetonitrile, vortexed briefly and centrifuged at 3500*g* for 3 min. Brain tissues were homogenized, mixed with 1.5 mL acetonitrile, vortexed and centrifuged at 3500*g* for 3 min. Pellet and supernatant were separated and counted for radioactivity using the gamma counter. An aliquot (500 μL) of the supernatant obtained from the plasma and brain extractions were subjected to RP-HPLC analysis. RP-HPLC analysis was performed using a reversed phase C_{18} HPLC column (Econosphere C_{18} 250 × 10 mm, 10 μm) attached to a precolumn (Alltima C_{18} 33 × 7 mm, 10 μm) with 80:20 (v/v) MeOH/sodium acetate buffer (0.02 M, pH 5.5) as solvent system at a flow rate of 6 mL/min. The same treatment regiments as for the biodistribution studies were investigated.

To determine recovery capabilities of [^{11}C]MC80, as well as, the stability of the radiotracer during the workup, control experiments (n = 3) were done using plasma and brain spiked with 3 MBq (81 μCi) authentic [^{11}C]MC80. Sample workup was identical as described above. Results are expressed as percentages of the total activity ± SD.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.097. These data include MOL files and InChiKeys of the most important compounds described in this article.

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