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^{11}C-SAM: a new potential agent for prostate cancer diagnosis

Zoppolo F; Buccino P; Porcal W; Oliver P; Savio E; Engler H.
Centro Uruguayo de Imagenología Molecular, Montevideo, Uruguay

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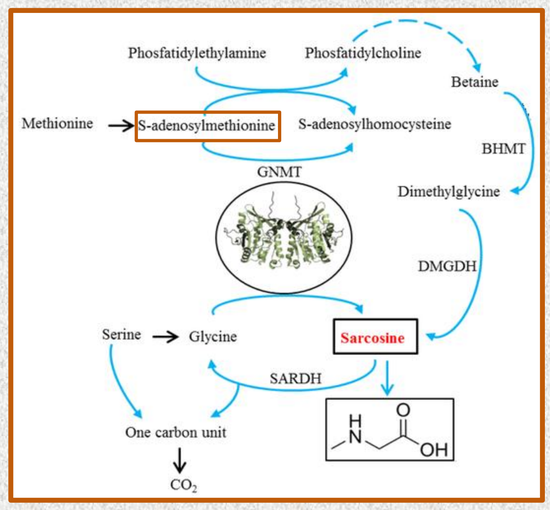
BACKGROUND

Prostate cancer (PCa) is one of the most common solid cancers in men. In patients with localized PCa, operative therapy is effective but a substantial number of patients experience recurrent disease. ^{11}C-choline (^{11}C-COL) has proved to be useful for restaging PCa in patients that suffer from biochemical failure with an absolute PSA value of > 1 ng/mL. This tracer cannot be recommended as a first-line screening procedure for primary PCa due to its limited sensitivity, its dependency on tumour configuration and its limited specificity in differentiation between PCa and benign pathologies.

Sarcosine (N-methyl glycine) has been identified as a differential metabolite that is highly increased during PCa progression to metastasis. This increase is associated to high levels of glycine N-methyltransferase (GNMT) in aggressive PCa. GNMT catalyzes the methylation of glycine using S-adenosylmethionine (SAM) as co-enzyme to form sarcosine.

The aim of this study is to identify GNMT ligands as potential radiotracers for aggressive PCa diagnosis. For this purpose SAM was labelled with ^{11}C and biological evaluation was performed. ^{11}C-Choline was selected as the reference radiopharmaceutical.



* Cernei N, et al. Int. J. Mol. Sci. 2013; 14:13893-13908.

METHODOLOGY

- **Synthetic strategies for ^{11}C-SAM**

The synthetic process begins with the production of ^{11}C-CO_2 in cyclotron (GE PETtrace 16.5 MeV) via the ^{14}N(p, α)^{11}C nuclear reaction. ^{11}C-CO_2 is delivered from the target to the automated synthetic platform (GE) TRACERlab® FX CPro, where it is trapped for purification and further reduction to ^{11}C-CH_4. Then, it is iodinated to yield ^{11}C-CH_3I, having the possibility to be later converted into ^{11}C-CH_3OTf. Finally, the labelling reactions are based on ^{11}C-methylations (figure 1).

$$^{11}C\text{-}CO_2 \xrightarrow[350ºC]{H_2\ (g)\ /\ Ni} {}^{11}C\text{-}CH_4 \xrightarrow[720\ ºC]{I_2\ (g)} {}^{11}C\text{-}CH_3I \xrightarrow[190\ ºC]{AgOTf} {}^{11}C\text{-}CH_3OTf$$

Figure 1: Left: GE TRACERlab FX CPro. Right: production of methylating agent.

In order to obtain ^{11}C-SAM, different labelling conditions of precursor (S-adenosyl homocysteine, SAH) have been performed, (figure 2). In these assays ^{11}C-CH_3I, ^{11}C-CH_3I/AgOTf and ^{11}C-MeOTf have been tested as methylating agents. TFA/H_2SO_4, formic acid/DMF and formic acid as solvents. The tested reaction temperatures were: 40, 60, 80, 100 and 120 ºC. Reaction times were: 1, 5, 10 and 30 min and the amount of precusor were 1, 3 and 5 mg.

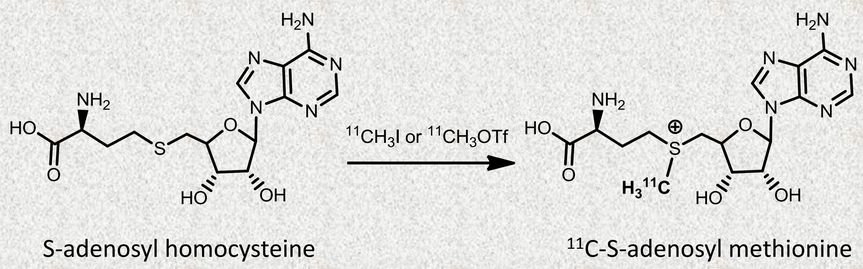


Figure 2: Synthetic strategies for ^{11}C-SAM.

Crude product from the reactor was separated from impurities using semipreparative HPLC (Phenomenex Luna C18 5µ 250x10 mm; buffer sodium acetate 0,1M (pH 4,5):MeCN (98:2); 6,0 mL/min). Fraction containing ^{11}C-SAM was collected over water and purified through Strata XC-SPE (Phenomenex), eluted with Na_2HPO_4 0,1M (pH 8,5):EtOH (9:1) and formulated in saline. The final product was filtered through a 0,22µm sterile membrane. ^{11}C-SAM quality control was performed evaluating according to the following parameters: solution appearance, pH, residual solvents (GC), chemical and radiochemical purity (HPLC), radionucleidic purity and identity, and specific activity.

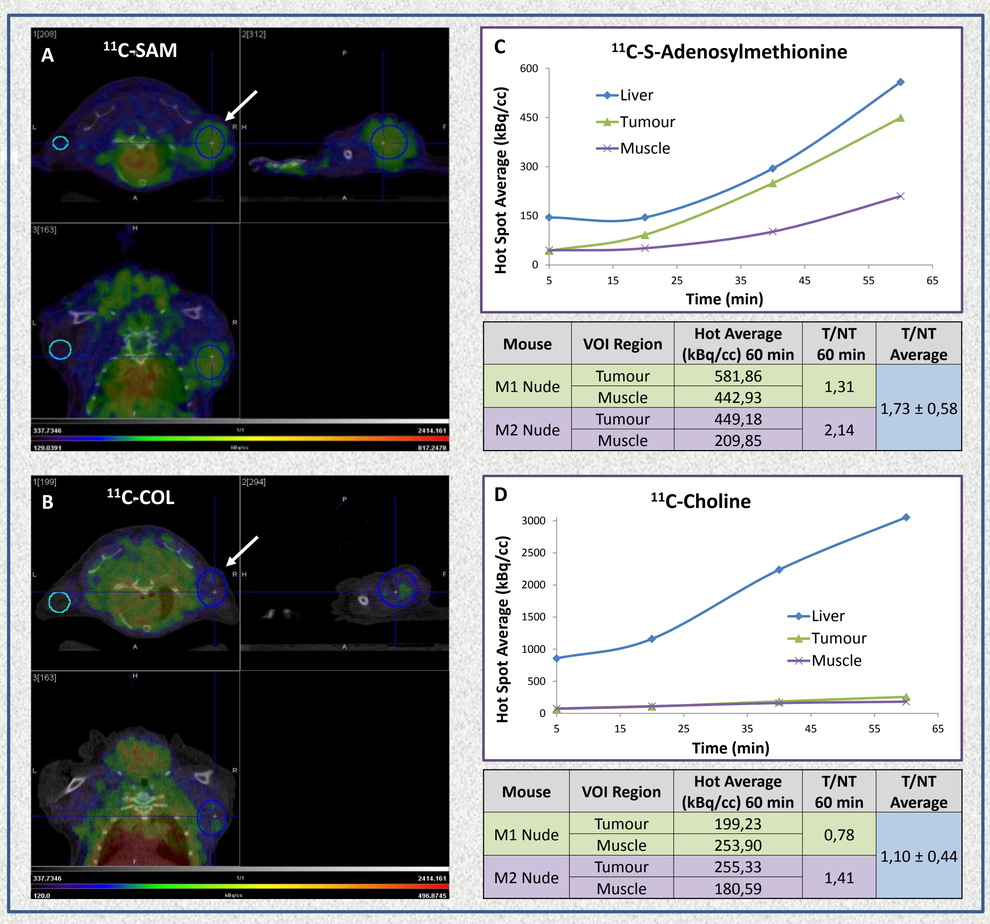
- **Preliminary Biological Studies of ^{11}C-SAM**

A xenographic human prostate cancer model was produced in two male nude mice. PC-3 cells (3 millions) were subcutaneously injected at the right upper leg of each animal. Appropiate tumour sizes (4,5-7,8 mm diameter) were achieved at four weeks post inoculation. Dynamic PET/CT scans were performed during 60 min in a preclinical Triumph Tri-modality Scanner (Trifoil, Inc.) after iv injection of ^{11}C-SAM (21,9-23,1 MBq) or ^{11}C-Choline (18,3-20,1 MBq) through the tail vein. Volumes of interest (VOIs) were drawn over tumour, contralateral muscle and liver to generate time-activity curves. The radioactivity concentration within each VOI was expressed as the Hot Spot Average (5) (kBq/cc). Biodistribution studies were carried out at 60 min post injection of ^{11}C-SAM (10,7-12,4 MBq). Results were expressed as percentage of activity per gram (% Act/g).

RESULTS

The optimum conditions for the labelling reaction were: methylating agent: ^{11}C-CH_3OTf, precursor amount: 5mg in formic acid, temperature: 60 ºC and reaction time: 1 min. With this conditions ^{11}C-SAM was obtained as a racemic mixture with a radiochemical purity of (97,4 ± 0,6)% and specific activities of (206,7 – 736,5)GBq/µmol.

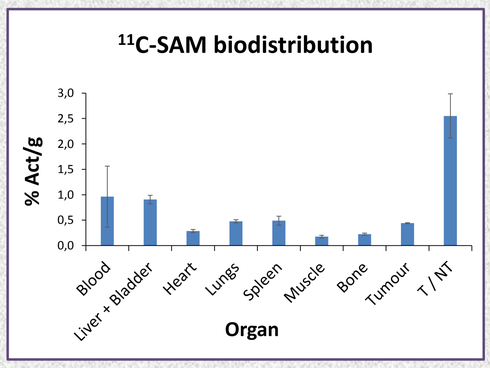
The PET/CT studies with ^{11}C-SAM showed an increased specific tumour uptake over contralateral muscle (T/NT) along time, which was higher than ^{11}C-COL. At 60 minutes of acquisition T/NT values were 1.73 ± 0.58 for ^{11}C-SAM and 1.10 ± 0.44 for ^{11}C-COL (figure 3). In biodistribution studies ^{11}C-SAM displayed a similar T/NT profile as for image studies with an average of 2.55 ± 0.44. High renal excretion of this tracer was observed which is in concordance with the high hydrophilicity of the compound, (figure 4).



Mouse	VOI Region	Hot Average (kBq/cc) 60 min	T/NT 60 min	T/NT Average
M1 Nude	Tumour	581,86	1,31	1,73 ± 0,58
	Muscle	442,93		
M2 Nude	Tumour	449,18	2,14	
	Muscle	209,85		

Mouse	VOI Region	Hot Average (kBq/cc) 60 min	T/NT 60 min	T/NT Average
M1 Nude	Tumour	199,23	0,78	1,10 ± 0,44
	Muscle	253,90		
M2 Nude	Tumour	255,33	1,41	
	Muscle	180,59		

Figure 3: PET/CT images of PC-3 tumour bearing nude mice injected with ^{11}C-SAM (A) and ^{11}C-COL (B) at 60 minutes of acquisition (1x5 min, 1x15 min, 2x20 min). Time-activity curves of PC-3 tumour with ^{11}C-SAM (C) and ^{11}C-COL (D).



* Kidneys % Act/g: (12,3 ± 1,8)

Mouse	Organ	% Act/g 60 min	T/NT 60 min	T/NT Average
M1 Nude	Tumour	0,44	2,24	2,55 ± 0,44
	Muscle	0,19		
M2 Nude	Tumour	0,45	2,86	
	Muscle	0,16		

Figure 4: Biodistribution results of PC-3 tumour bearing nude mice, administrated with ^{11}C-SAM, at 60 minutes post injection.

CONCLUSION

It was possible to optimize the ^{11}C-SAM radiosynthesis, obtaining a tracer that is within the quality control specifications. The preliminary biological studies showed higher T/NT ration for ^{11}C-SAM compared to ^{11}C-COL, indicating GNMT ligands seem to be very promising compounds for prostate cancer diagnosis. Further studies must be performed in order to obtain a deeper and complete biological characterization of this compound.

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