GNMT ligands and sarcosine related metabolites as potential tracers for prostate cancer diagnosis

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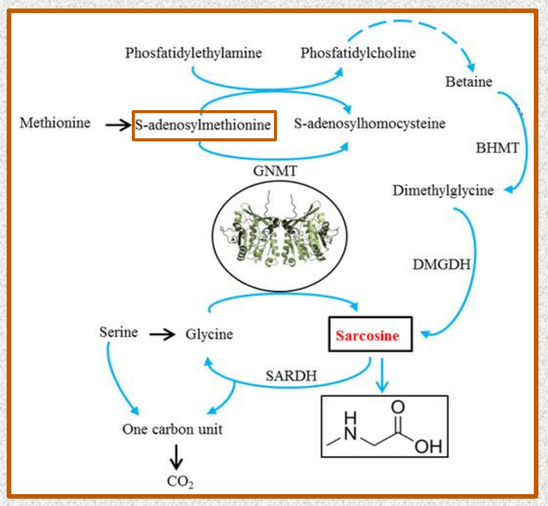
INTRODUCTION

Prostate cancer (PCa) is one of the most common solid cancers in men. Its incidence in Uruguay is 60,7/100.00, being the second cause of mortality (20,6/100.00) for cancer. ^{11}C-Choline has proved to be useful for restaging PCa in patients who suffer from biochemical failure with an absolute PSA value of > 1 ng/mL. The limited sensitivity, the dependency on tumour configuration and low specificity in differentiation between PCa and benign pathologies are important reasons to search for new radiotracers. Sarcosine has been identified as a metabolite highly increased during PCa in aggressive progression to metastasis, having and intermediary role in neoplasic progression. This increase is associated to high levels of glycine N-methyltransferase (GNMT), enzyme that catalyzes the methylation of glycine using S-adenosylmethionine (SAM) as co-enzyme to form sarcosine.



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

AIM

The aim of this study is the development of new Positron Emission Tomography (PET) radiotracers that contributes to PCa diagnosis, evaluates its aggressivity and progression using molecular imaging. ^{11}C-Choline was taken as reference. GNMT ligands have been identified and SAM was selected as one option to be labelled with ^{11}C.

OBJECTIVES

- Synthesis of SAM
- Synthetic strategies for ^{11}C-SAM
- Study and evaluation of others GNMT ligands and metabolites involved in the sarcosine pathway as potential biomarkers.
- Labelling strategies for selected molecules with PET radionuclides.
- Biological characterization of labelled molecules by *in vitro* and *in vivo* studies.

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 C Pro, 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 C Pro. 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 120º C. Reaction times were: 1, 5, 10 and 30 min and the amount of precusor were 1, 3 and 5 mg.

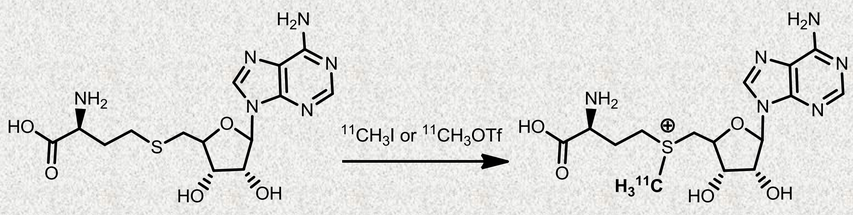


Figure 2: Synthetic strategies for ^{11}C-SAM.

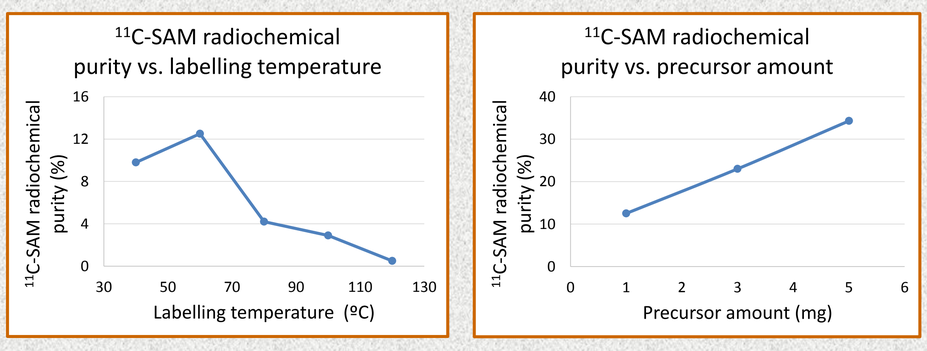


Figure 3: Variations of ^{11}C-SAM radiochemical purity in the reaction mixture with different labelling temperatures and precursor amounts.

The optimum conditions for the labelling reaction are summarized in figure 4. ^{11}C-SAM was obtained with a radiochemical purity of 32% in the reactor, as a mixture of two stereoisomers, as revealed by the double peak in the gamma chromatogram.

Methylating agent	Solvent	Precursor amount	Labelling temperature	Reaction time	^{11}C-SAM radiochemical purity
$^{11}CH_3OTf$	Formic acid	5mg	60ºC	1 min	32%

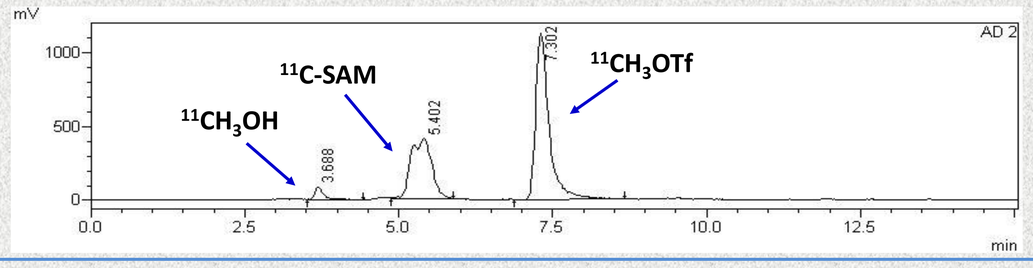


Figure 4: Optimum labelling conditions and gamma chromatogram of the reaction mixture.

SAM has a chiral sulfur center found in two enantiomeric forms, making SAM a diastereomer: (S,S)-SAM and (R,S)-SAM, (Figura 5). The (S,S) configuration is the only biosynthesized form and the biologically active form for all SAM dependent methyltransferases. Therefore, it would be really useful to optimize an efficient chromatographic method to separate the isomers obtaining (S,S)-SAM pure.

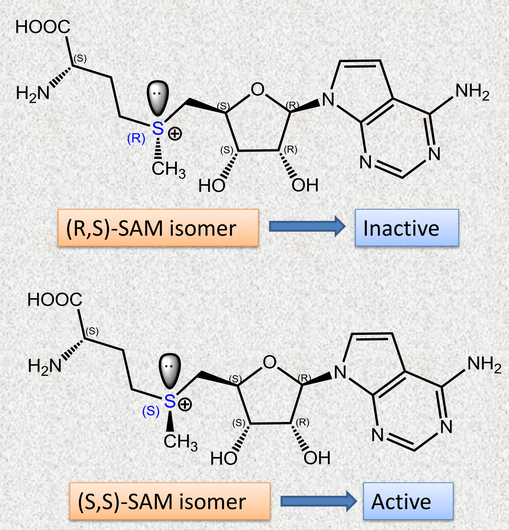
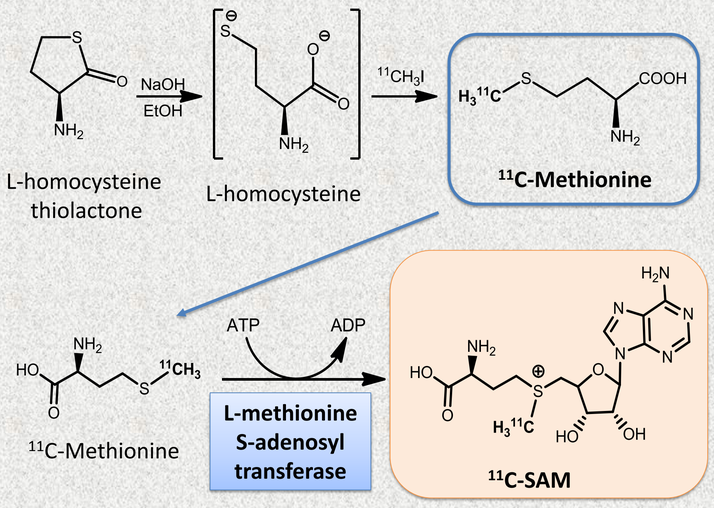


Figure 5: (S,S)- and (R,S)- forms of SAM.

OUTLOOK



To improve the radiochemical purity of the (S,S)-isomer, an enzymatic labelling of ^{11}C-SAM will be performed as explained in figure 6. (Ishiwata K, et al. Eur. J. Nucl. Med. 1986; 11: 449-452).

Figure 6: Left: Enzymatic synthesis of SAM.

Another GNMT ligands and metabolites involved in the sarcosine pathway (glycine and methionine), are also considered as potential biomarkers for detecting PCa progression and aggressivity. Biological characterization of new labelled compounds will be performed in future studies including: a) dynamic PET/CT scans in nude mice (control as well as xenographic human PCa-bearing tumours) in a preclinical Triumph Tri-modality Scanner (Gamma Medica, Inc.); b) biodistributions in the same nude mice models at 10, 20 and 40 min; c) cellular internalization in PC3, VCap and LNCap human tumour lines.

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