Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVIII, No. 7

SYNTHESIS OF [2-^{14}C]THYMIDINE : A POTENTIAL ROUTE FOR [2-^{11}C]THYMIDINE

T. VANDER BORGHT*, S. PAUWELS**, L. LAMBOTTE***, C. DE SAEGER*, C. BECKERS**
Departments of Gastroenterology*, Nuclear Medicine** and Experimental Surgery***,
University of Louvain Medical School, Brussels, Belgium

SUMMARY

We report a three-step synthesis of thymidine, starting from trace amounts of urea, that allows rapid introduction of radioactivity in C2-position of the pyrimidine ring. Thymine was produced within 5 min with a 60 % yield by cyclocondensation of urea and diethyl ß-methylmalate in fuming sulfuric acid (15% SO_3). Thymidine was further synthesized by the enzymatic transfer of deoxyribose onto thymine with a 90 % yield achieved within 10 min. Using [^{14}C]urea as precursor, the present method allowed the synthesis of radiochemically pure [2-^{14}C]thymidine in 35 min with a cumulative yield of 40 %.

KEYWORDS: thymidine, thymine, carbon-14, carbon-11, PET, liver growth.

INTRODUCTION

Although tumor growth has been successfully studied by positron emission tomography and [methyl-^{11}C]thymidine (1), the method appeared not specific enough to quantitate hepatic cellular proliferation (unpublished data). Using thymidine labelled with ß-emitters, we have shown that, when thymidine labelled in C2-position was used instead of methyl-labelled thymidine, the labelled metabolites were rapidly eliminated in the form of CO_2, so that the intrahepatic radioactivity reflected almost exclusively the incorporation pathway of thymidine into DNA (2).

Synthesis of [2-^{11}C]thymidine has never been reported and none of the methods proposed for preparation of cold or [2-^{14}C]labelled thymidine (3-6) are transposable to carbon-11 radiochemistry. In the light of recent findings that [^{11}C]urea can be synthesized with high yields within 15 min (7), we evaluated a three-step synthetic approach starting with trace amounts of cold and ^{14}C-labelled urea and short reaction times that could constitute a potential route for production of [2-^{11}C]thymidine.

Address for reprint requests : Dr. S. Pauwels, Centre de Médecine Nucléaire, UCL 54.30, Avenue Hippocrate, 54, B-1200 Brussels, Belgium.

0362-4803/90/070819-04$05.00

Received December 4, 1989
Revised January 24, 1990

MATERIAL AND METHODS

Chemical reagents. Cold urea, P_2O_5, H_3PO_4, sulfuric acid, fuming sulfuric acid (30% SO_3) were purchased from Merck (Darmstadt, FRG). Citraconic acid (methylmaleic acid), thymine, thymidine, 2'-deoxyribose-1-phosphate and thymidine phosphorylase were obtained from Sigma (St Louis, MO, USA), [^{14}C]urea and [2-^{14}C]thymidine from Amersham (Little Chalfont, UK). Diethyl ß-methylmalate (diethyl 2-hydroxy-2'-methylsuccinate) was prepared by hydrogenation of diethyl 2-methyl-2'-oxosuccinate (Janssen, Beerse, Belgium) according to the method of Sherp (8). ß-Methylmalic acid was produced by acidic hydrolysis of diethyl ß-methylmalate.

Synthesis of 2C-labelled thymidine. Anhydrous urea (5 µmol) dissolved in 0 to 35 µl absolute ethanol was mixed with 20 to 100 µmol diethyl ß-methylmalate in fuming sulfuric acid (0 to 30% SO_3). The mixture was heated during 5 to 20 min at 60 to 150°C. The reaction was quenched by cooling at 0°C. The mixture was further diluted with water and neutralized with 5M NaOH prior to HPLC analysis. The structure of synthesized thymine was also confirmed by mass spectroscopy. All experiments were tested 3 times and results expressed as mean ± SD.

The enzymatic transfer of deoxyribose onto thymine was performed by a modification of the method of Filip (5,9). One µmol of thymine was incubated for 10 min with 2'-deoxyribose-1-phosphate and thymidine phosphorylase in 1.0ml 100 mM Tris-HCl pH 7.4. The glycosylation of thymine was tested under varying conditions of pH (6.6 to 8.6), temperature (30-65°C), enzymatic concentration (1-40 I.U.), substrates molar ratio (deoxyribose:thymine ratio 1:1 to 1:5) and Na_2SO_4 concentration (0.2 to 2.0 M). The synthesized material was characterized by HPLC.

HPLC analysis. The reaction mixtures obtained after cyclocondensation and after the enzymatic reaction were applied to a RP-18 column (Serva, 4.6x250mm, 10 µm). Columns were eluted with a linear gradient from 15% to 35% methanol over a period of 5 min at the constant flow rate of 2 ml.min^{-1}, and eluates were monitored by A_{280}. Columns were calibrated with standards of thymine and thymidine. The yield of syntheses was calculated on basis of the initial amount of urea and thymine used in the cyclocondensation and glycosylation steps, respectively.

RESULTS AND DISCUSSION

Most of the procedures described for synthesis of pyrimidine derivatives by condensation reactions starting with urea necessitate very long heating periods in presence of fuming sulfuric acid or polyphosphoric acid (PPA) (3,4,6,8,10). In pilot experiments, no chromatic evidence of thymine was obtained when PPA was heated for 20 min in presence of a mixture of urea and citraconic acid, ß-

methylmalate or diethyl ß-methylmalate (fig.1). In contrast, the reaction proceeded in presence of fuming sulfuric acid and a yield of 64.4±2.1 % was obtained by reaction of 5 µmol urea, dissolved in 20 µl ethanol, with 50 µmol diethyl ß-methylmalate when the mixture was heated for 10 min at 130°C in 0.1ml fuming sulfuric acid (15% SO_3). The solubilization of urea was critical for the yield of the synthesis, highest yields being achieved when 5 µmol urea were dissolved in 20-25 µl ethanol prior to addition of diethyl ß-methylmalate and fuming sulfuric acid. Increasing the temperature from 60 to 110°C had the most pronounced effect of the factors tried, with a yield increasing from 2 to 60 %. A progressive improvement in yield was obtained when the ratio urea:diethyl ß-methylmalate was increase from 1:4 to 1:10. In these optimized conditions, yields were not significantly improved by reaction times exceeding 5 min.

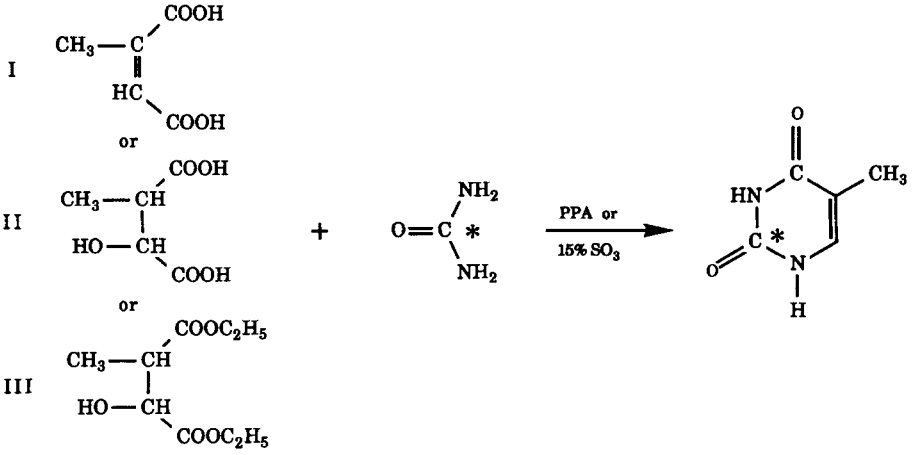

Figure 1. Alternative reagents used for cyclocondensation with urea to produce thymine (I: citraconic acid; II: ß-methylmalic acid; III: diethyl ß-methylmalate).

Transformation of thymine to thymidine averaged 90 % when thymine and 2'-deoxyribose-1-phosphate (ratio 1:3) were incubated for 10 min at 30-50°C in presence of 5 I.U. thymidine phosphorylase. Increasing the amount of enzyme above 20 I.U. resulted in a progressive reduction of the reaction yield. Keeping in mind that synthesis of thymine is achieved in fuming sulfuric acid, further experiments were performed in order to evaluate the effect of the pH and the ionic strength on the yield of the enzymatic reaction. The yield of the reaction dropped off rapidly with Na_2SO_4 concentrations exceeding 1M whereas pH was not critical when maintained between 6.6 and 8.6.

Using the optimized conditions, [2-^{14}C]thymidine was produced with a yield of 40 % and a synthesis and purification time of 35 min. [2-^{14}C]Thymine was

synthesized starting with a mixture of 1 µmol [^{14}C]urea and 4 µmol carrier in 20µl absolute ethanol that was allowed to react at 130°C for 5 min with 50 µmol diethyl ß-methylmalate in 0.1ml fuming sulfuric acid (15 % SO_3). The pH was neutralized by addition of 5M NaOH and Tris-HCl buffer. The reaction mixture was desalted using a AG 11A8 ion retardation resin (Bio-Rad, Richmond, CA, USA) and further incubated at 40°C for 10 min with 3 µmol 2'-deoxyribose-1-phosphate and 5 I.U. thymidine phosphorylase. The sample was purified by HPLC and the eluate containing the [2-^{14}C]thymidine was evaporated, resuspended in 0.15 M NaCl and sterilized by ultrafiltration. Biodistribution studies in rats showed a hepatic uptake and metabolism of the newly synthesized [2-^{14}C]thymidine comparable to authentic [2-^{14}C]thymidine.

The use of a simple precursor and the high yields obtained with trace amounts of reagents in an acceptable time span make the method attractive for carbon-11 radiochemistry. Since [^{11}C]urea can be produced from [^{11}C]CN^- within 16 min from end of bombardment with a radiochemical yield of 95 %, the synthesis and purification of [2-^{11}C]thymidine should be achieved within 60 min with a decay corrected radiochemical yield of 35 %.

ACKNOWLEDGEMENT

This work has been supported by a Grant N°3.4554.85 and N°9.4510.88 from the Fund for Medical Scientific Research (Belgium). The National Fund for Scientific Research provided a Grant support to Dr. T. Vander Borght (Research Assistant). The authors thank Dr. D. Labar for his helpful discussion.

REFERENCES

1. Martiat P., Ferrant A., Labar D., Cogneau M., Bol A., Michel C., Michaux J.L., Sokal G. - J. Nucl. Med. 29: 1633 (1988)
2. Vander Borght T., Pauwels S., Lambotte L., Beckers C. - J. Nucl. Med. 30: 929 (1989)
3. Bennett L, Jr - J. Am. Chem. Soc. 74: 2432 (1952)
4. Fel'dman I., Shepshelevich L. - Chem. Abst. 59: 7527 (1962)
5. Filip J., Nejedly Z. - J. Lab. Comp. 3: 128 (1967)
6. Jezdic V., Razumenic N., Skakun M., Albahari S.J., Odavic-Josic J. - J. Lab. Comp. 6: 88 (1970)
7. Emran A., Boothe T., Finn R., Vora M., Lothari P. - Int. J. Appl. Radiot. Isot. 36: 739 (1985)
8. Sherp H. - J. Am. Chem. Soc. 68: 912 (1946)
9. Filip J., Pritasil L., Nejedly Z., Vesely J., Cihak A. - J. Lab. Comp. 14: 297 (1977)
10. Harada K., Suzuki S. - Tetrahedron Lett. 27: 2321 (1976)