



DEVELOPMENT OF A POSITRON EMISSION TOMOGRAPHY RADIOPHARMACEUTICAL FOR IMAGING THYMIDINE KINASE GENE EXPRESSION: SYNTHESIS AND IN VITRO EVALUATION OF 9-[(3-[¹⁸F]FLUORO- 1-HYDROXY-2-PROPOXY)METHYL]GUANINE

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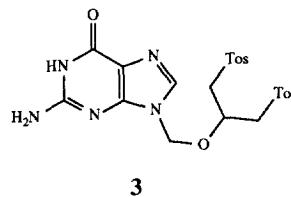
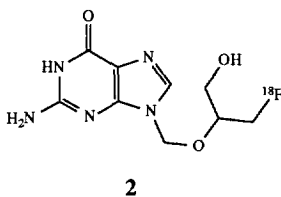
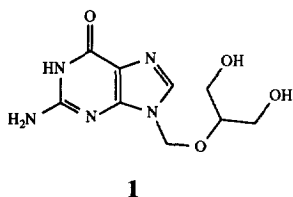
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Abstract: 9-[(3-fluoro-1-hydroxy-2-propoxy)methyl]guanine (FHPG) **2** has been labeled with fluorine-18 and evaluated *in vitro* as a potential radiotracer for mapping gene expression *in vivo* with positron emission tomography (PET). © 1997 Elsevier Science Ltd.

Recently new approaches of gene therapy have been proposed for the treatment of various human diseases. Among them, a promising gene therapy protocol for cancer consists of the transduction of neoplastic cells with the herpes simplex virus thymidine kinase gene (HSV-tk) which renders transduced cells sensitive to the lethal effect of antiviral agent such as ganciclovir (DHPG) **1**.¹

Positron emission tomography (PET) with adapted radiotracers represents a potential tool to determine the *in vivo* level of HSV-tk expression and to establish the optimal protocol of gene and ganciclovir administration in human. Until now, only a few molecules with potential applications to PET have appeared in the literature. 5-Iodo-2'-fluoro-2'-deoxy-1-β-D-arabinofuranosyl-5-iodouracil (FIAU)^{2,3} and 8-fluoro-9-[(2-hydroxyethoxy)methyl]guanine⁴ have been investigated as possible candidates for PET studies. Synthesis of 9-[(3-[¹⁸F]fluoro-1-hydroxy-2-propoxy)methyl]guanine **2** was recently achieved^{5,6} and we report here our preliminary results with this molecule. Acyclic guanosine analogs are known to be potent antiviral agents against the herpes virus. Martin *et al.* first described FHPG and showed that it was less active than ganciclovir against herpes simplex virus 1 (HSV-1) in *in vitro* assays but none the less remained a good substrate for viral thymidine kinase.⁷



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Radiosynthesis of **2** was achieved by nucleophilic [^{18}F]fluorination of the ditosylate **3**⁸ followed by a basic hydrolysis and HPLC purification with a 3% (decay corrected) radiochemical yield.

In a typical experiment, anhydrous potassium [^{18}F]fluoride/K222⁹ was added to a suspension of **3** in acetonitrile. The solution was refluxed for 30 minutes. The acetonitrile mixture was diluted with water (20 mL) and the whole solution was passed through an activated C18 Sep Pak[®] column (Waters). The column was washed with 10 mL of 1 M HCl, 10 mL of water and the activity was eluted with 2 mL of ethanol. The solvent was concentrated and 1 mL of 1 M KOH was added. The solution was refluxed for 30 minutes, cooled and acidified with 1 M HCl until pH 1.5. The resulting solution was injected on a semi-preparative HPLC column (C8, 10 μm , 10 X 250 mm; eluent 50 mM NH_4OAc , 4% CH_3CN ; flow rate 7 mL/min, UV detection: $\lambda = 254 \text{ nm}$). The appropriated radioactive fraction was collected at about 12 minutes, acetonitrile was removed under reduced pressure and the isotonicity of the solution was adjusted with sodium chloride. The purified radioactive compound was analyzed by HPLC (C18, 5 μm , 4.6 X 250 mm; eluent 50 mM NH_4OAc , 4% CH_3CN ; flow rate 1 mL/min, UV detection: $\lambda = 254 \text{ nm}$) and was coeluted with an authentic sample which was synthesized using the procedure described by Martin *et al.*⁷ For *in vivo* experiments the solution may be sterilized by passing through a 0.22 μm filter membrane (Cathivex[®], Waters) before isotonicity adjustment with sterile sodium chloride.

The cytotoxic effect of FHPG, DHPG and acyclovir on cell growth was evaluated on cells maintained in culture. The cell lines used have been described elsewhere.¹⁰ In short, the 9L cell line is a chemically induced gliosarcoma from the Fischer 344 rat strain. The 9Ltk+ cells were obtained by transfer of the herpes simplex type 1 thymidine kinase and the neomycine resistance gene. The 9Ltk- cells were obtained by transfer of the neomycine resistance gene. This procedure was described by Cool *et al.*,¹⁰ where 9Ltk+ refers to 9L transduction with the pTCM1-LTKSN vector and 9Ltk- refers to 9L transduction with the pLXSN vector.

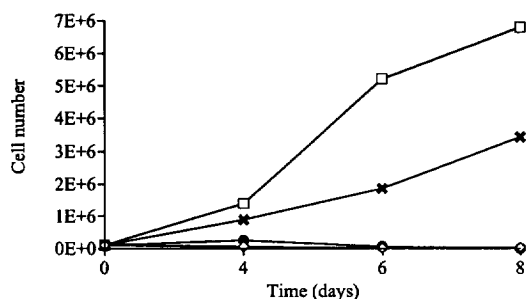


Figure 1. Effect of FHPG, DHPG and acyclovir on cell growth. Cells (9Ltk+) were incubated with no drug as control (□), with FHPG at 100 μM (*), with DHPG at 10 μM (●) or with acyclovir at 100 μM (◇) for 4, 6 and 8 days.

In 9Ltk+ cells, the cytotoxic effects of FHPG was evaluated and compared to those of two well established antiviral drugs: acyclovir and DHPG. FHPG at 100 μM inhibits the cell growth by 50% while DHPG at 10 μM and acyclovir at 100 μM completely stopped the process (Fig. 1). These data were in good agreement with those described by Martin *et al.*⁷ It is noteworthy that we were using for this test the racemic mixture of FHPG and that pure enantiomers might have different antiviral activities.¹¹⁻¹⁴

Phosphorylated forms of [^{18}F]FHPG should theoretically accumulate in cells expressing HSV-tk. We have tested the *in vitro* uptake of no-carrier-added (n.c.a.) [^{18}F]FHPG in 9Ltk+ and 9Ltk- cells. On day 0, 2.5×10^6 9Ltk+ or 9Ltk- cells were seeded in Petri dishes in duplicate for each experimental group and timing. On day 2, [^{18}F]FHPG (7.4 MBq) was added in each dish. After 30, 60, 120, 240 and 360 minutes of incubation at 37°C under a CO_2 and water controlled atmosphere, culture medium was discarded and cells were washed 3 times with 5 mL of culture medium and harvested after trypsinization. All samples were counted in a γ spectrometer. Accumulation of [^{18}F]FHPG in

9Ltk+ and 9Ltk- cells is shown in Figure 2. In two different experiments, the radioactivity ratio in 9Ltk+ and 9Ltk- cells (corrected for the number of cells) was respectively 234 and 243 after 360 minutes of incubation.

The above cell pellets were treated with 300 μ L of 1% sodium dodecyl sulfate and 80 μ L of 3% perchloric acid. After centrifugation, 250 μ L of the supernatant were then added to 250 μ L of HPLC solution (KH_2PO_4 0.01 M; pH 3.5). The extracts (100 μ L) were analyzed by HPLC with a Whatman Partisil SAX column (10 μ m, 4.6 X 250 mm, UV detection: $\lambda=254$ nm).¹⁵ The column was eluted at a flow rate of 1 mL/min with a linear gradient of KH_2PO_4 , 0.01 to 1 M (pH 3.5). A 30 minutes gradient was followed by 15 minutes at 1 M buffer to elute the triphosphate. Fractions of 2 mL were collected and assayed for radioactivity by γ spectrometry. HPLC retention times of [^{18}F]FHPG and [^{18}F]FHPG monophosphate were 6 and 10 minutes respectively.¹⁶ Analysis of [^{18}F]FHPG metabolites in

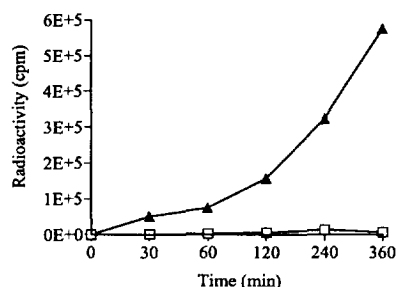


Figure 2. Accumulation of [^{18}F]FHPG in 9Ltk+ cells (▲) and 9Ltk- (□).

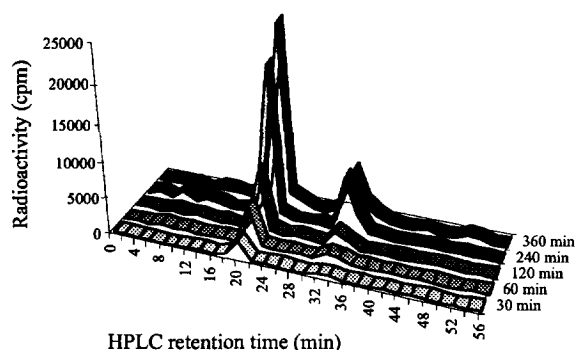


Figure 3. HPLC analysis of the metabolites of [^{18}F]FHPG in 9Ltk+ cells after 30, 60, 120, 240 and 360 min of incubation.

As a matter of fact, Figure 3 shows that the relative amount of the different chemical species remains approximately the same from 30 to 360 minutes after exposure to [^{18}F]FHPG, with a clear prevalence of two more polar metabolites beside [^{18}F]FHPG monophosphate. These observations are in good agreement with those previously reported.¹⁷

In the same conditions, retention time of [^3H]DHPG mono-, di- and triphosphate peaks are 11, 19 and 32 min, respectively; [^3H]acyclovir mono-, di- and triphosphate peaks eluted at 11, 21 and 35 minutes, respectively. If, as suspected, the more polar metabolite is the [^{18}F]FHPG triphosphate, and since this form should be the active one for DNA polymerase inhibition, we may postulate that the minor production of [^{18}F]FHPG triphosphate (25%) in our system explains that we found weak anti-proliferation activity of FHPG.

These observations prompt us to conclude that [^{18}F]FHPG is a promising PET tracer for the evaluation of gene therapy involving thymidine the kinase gene.

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References and Notes

1. Culver, K. W.; Ram, Z.; Wallbridge, S.; Ishii, H.; Oldfield, E. H.; Blaese, R. M. *Science* **1992**, *256*, 1550.
2. Tjuvajev, J. G.; Stockhammer, G.; Desai, R.; Uehara, H.; Watanabe, K.; Gansbacher, B.; Blasberg, R. G. *Cancer Res.* **1995**, *55*, 6126.
3. Tjuvajev, J. G.; Finn, R.; Watanabe, K.; Joshi, R.; Oku, T.; Kennedy, J.; Beattie, B.; Koutcher, J.; Larson, S.; Blasberg, R. *Cancer Res.* **1996**, *56*, 4087.
4. Barrio, J. R.; Namavari, M.; Phelps, M. E.; Satyamurthy, N. *J. Org. Chem.* **1996**, *61*, 6084.
5. Monclus, M.; Luxen, A.; Van Naemen, J.; Damhaut, P.; Velu, T.; Cool, V.; Goldman, S. *J. Labelled Compd Radiopharm* **1995**, *37*, 193.
6. Alauddin, M. M.; Conti, P. S.; Mazza, S. M.; Hamzeh, F. M.; Lever, J. R. *Nuclear Medicine & Biology* **1996**, *23*, 787.
7. Martin, J. C.; McGee, D. P. C.; Jeffry, G. A.; Hobbs, D. W.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1986**, *29*, 1384.
8. Preparation of 9- $\{[1,3\text{-di}(p\text{-toluenesulfonyloxy})\text{-2-propoxy}]\text{methyl}\}$ guanine **3**. To a suspension of DHPG **1** (255 mg, 1 mmole) in anhydrous pyridine (2 mL) was added with a syringe *p*-toluenesulfonyl chloride (955 mg, 5 mmoles) previously dissolved in anhydrous pyridine (1 mL). After addition, the suspension was allowed to stand at room temperature for 12 h. The solution was then poured in 1 M HCl/ice and then filtered. The crude material was purified by using column chromatography (SiO₂, CH₂Cl₂/methanol: 9/1 and then CH₂Cl₂/methanol: 8/2) to afford pure ditosylate **3** (230 mg, 40%) as a solid mp 272-273°C (dec). ¹H-NMR (250 MHz, DMSO-*d*₆): δ 2.41 (s, 6H, CH₃), 3.88 (dd, *J*=5.7, 10.6 Hz, 2H, CH₂ (5')), 4.02 (dd, *J*=3.5, 10.7 Hz, 2H, CH₂ (3')), 4.08-4.12 (m, 1H, CH (4')), 5.28 (s, 2H, CH₂), 6.57 (s, 1H, NH), 7.43 (d, *J*=8.25 Hz, 2H), 7.65 (d, *J*=7.8 Hz, 2H); HPLC-MS [M+H]⁺: 564.
9. Guillaume, M.; Luxen, A.; Nebeling, B.; Argentini, M.; Clark, J. C.; Pike, V. W. *Appl. Radiat. Isot.* **1991**, *42*, 749.
10. Cool, V.; Pirotte, B.; Gérard, C.; Dargent, J.-L.; Baudson, N.; Levivier, M.; Goldman, S.; Hildebrand, J.; Brotchi, J.; Velu, T. *Hum. Gen. Ther.* **1996**, *7*, 627.
11. Ashton, W. T.; Canning, L. F.; Reynolds, G. F.; Tolman, R. L.; Karkas, J. D.; Liou, R.; Davies, M.-E. M.; DeWitt, C. M.; Perry, H. C.; Field, A. K. *J. Med. Chem.* **1985**, *28*, 926.
12. Karkas, J. D.; Germershausen, J.; Tolman, R. L.; MacCoss, M.; Wagner, A. F.; Liou, R.; Bostedor, R. *Biochim. Biophys. Acta* **1987**, *911*, 127.
13. Kohlbrenner, W. E.; Carter, C. D.; Fesik, S. W.; Norbeck, D. W.; Erickson, J. *Biochem. Pharmacol.* **1990**, *40*, R5.
14. Synthesis of R and S enantiomers of FHPG. M. Monclus. Unpublished data.
15. Elion, G. B.; Furman, P. A.; Fyfe, J. A.; de Miranda, P.; Beauchamp, L.; Schaeffer, H. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5716.
16. An authentic sample of FHPG monophosphate was prepared by treating N-acetyl-FHPG with 2-cyanoethyl N,N-diisopropylchlorophosphoramidite. Conversion to the intermediate dicyanoethylphosphoramidite and subsequent oxidation, deprotection and purification by HPLC on a SAX column afforded the desired compound.
17. Smee, D. F.; Boehme, R.; Chernow, M.; Binko, B. P.; Matthews, T. R. *Biochem. Pharmacol.* **1985**, *34*, 1049.