PH.D. DISERTATION's THESES

PRODUCTION OF SOME [^{11}C]-LABELLED CAFFEINE DERIVATIVES AS POSSIBLE TRACERS FOR MAPPING ADENOSINE A_{2A} RECEPTORS BY PET

ISTVÁN BOROS

University of Debrecen
Faculty of Natural Sciences
DEBRECEN, 2002.

Introduction

The role of adenosine in modulating the function of the cardiovascular, endocrine, and nervous system has been known for several decades [Daly J. W., 1982]. Recently the adenosine/P_1 purinoceptors were classified into four subtypes: A_1, A_{2A}, A_{2B} and A_3 receptors [Jacobson K. A., 1993]. The subtype A_{2A} receptors are localised mainly in brain, their highest expression was found in the striatum, nucleus accumbens and tuberculum olfactorium, whereas the A_{2A} receptors showed wide distribution, with high expression in the gastrointestinal tract. Ligands having antagonist receptor effect are especially beneficial in promoting a more complete understanding of the role of different subtypes of receptors in physiological responses.

In vitro autoradiographic data indicate that some 8-styryl-caffeine derivatives are high affinity antagonists specific to A_{2a} type of adenosine receptors.

The goal of this work was to evaluate the reaction conditions in the synthesis of different (E)-8-styryl-7-[^{11}C]methyl-xanthine derivatives ([^{11}C]-caffeines) as possible tracers for adenosine receptor research using PET technique. An obvious route for radiolabelling of these compounds seems to be the N-methylation of the corresponding (E)-8-styryl-xanthine derivative using [^{11}C]-methyl iodide. Ten A_{2a} adenosine receptor specific (E)-8-styryl-7-[^{11}C]methyl-xanthine derivatives ([^{11}C]-caffeines) were synthesised by N-methylation of the corresponding (E)-8-styryl-xanthine derivatives using [^{11}C]-methyl iodide.

In vivo data indicate that 8-(3-chlorostyryl)caffeine (CSC) is a high affinity antagonist specific to A_{2a} type of adenosine receptors. The CSC is a selective ligand having higher affinity for A_2 receptors (K_i=54 nM) than for A_1 receptors (K_i=28200 nM). We chose (E)-8-(3-chloro-styryl)-1,3-dimethyl-7-[^{11}C]methyl-xanthine ([^{11}C]-

CSC) for biological studies and present an evaluation of its potential as a tracer for mapping adenosine A_{2a} receptors by positron emission tomography (PET).

Materials

The inactive standard compounds, (*E*)-8-styryl-1,3,7-trimethylxanthine (SC); (*E*)-8-(3-chlorostyryl)-1,3,7-trimethylxanthine (CSC); (*E*)-8-(3-iodostyryl)-1,3,7-trimethylxanthine (ISC); (*E*)-8-(3-nitrostyryl)-1,3,7-trimethylxanthine (NSC); (*E*)-8-(3,4-dimethoxystyryl)-1,3,7-trimethylxanthine (3,4-DMSC); (*E*)-8-(3,5-dimethoxystyryl)-1,3,7-trimethylxanthine (3,5-DMSC); (*E*)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine (TMSC); (*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine (3,4-DMDPrSC); (*E*)-8-(3,5-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine (3,5-DMPrSC) and the corresponding precursor compounds: (*E*)-8-styryl-1,3-dimethylxanthine (SX); (*E*)-8-(3-chlorostyryl)-1,3-dimethylxanthine (CSX); (*E*)-8-(3-iodostyryl)-1,3-dimethylxanthine (ISX); (*E*)-8-(3-nitrostyryl)-1,3-dimethylxanthine (NSX); (*E*)-8-(3,4-dimethoxystyryl)-1,3-dimethylxanthine (3,4-DMSX); (*E*)-8-(3,5-dimethoxystyryl)-1,3-dimethylxanthine (3,5-DMSX); (*E*)-8-(3,4,5-trimethoxystyryl)-1,3-dimethylxanthine (TMSX); (*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropylxanthine (3,4-DMDPrSX); (*E*)-8-(3,5-dimethoxystyryl)-1,3-dipropylxanthine (3,5-DMPrSX) were prepared following a previously described general procedure [Jacobson K. A., 1993], and were characterised by their physical data (mp., ^{1}H-NMR, HPLC). Nuclear magnetic resonance spectra were taken on a Bruker WP 200 spectrophometer (200 MHz) using Me_4Si as internal standard.

The 3-iodocinammic acid, used as intermediate in the above general procedure for synthesis of ISX, was prepared by reduction, diazotization, followed by iodination of m-nitrocinnamic acid [Patterson T.S., 1889]. Being new compounds the structures of (*E*)-8-(3-iodostyryl)-1,3,7-trimethylxanthine (ISC) (mp. 226-227°C) and (*E*)-8-(3-iodostyryl)-1,3-dimethylxanthine (ISX) (mp. >360°C) were proved by their ^{1}H-NMR spectra.

[^{11}C]Methyl iodide

[^{11}C]CO_2 was produced on the MGC 20E cyclotron at the Institute of Nuclear Research, Debrecen by the $^{14}N(p,\alpha)^{11}C$ ($^{11}C+O_2 \rightarrow {}^{11}CO_2$) nuclear reaction, with 14.5 MeV proton beam and $1.3x10^6$ Pa target gas pressure (the yield was 1.6-2.2 GBq/μA). [^{11}C]-Methyl iodide was prepared using an automated synthesis system. The specific activity of a typical 100 mCi [^{11}C]CH_3I batch was in the range of 1-1.5 Ci/μmol at 20 min after EOB.

General procedure of [^{11}C]-methylation

To prevent *E*-to-*Z* isomerization of the (*E*)-xanthines during the radiosynthesis all procedures were carried out in amber glassware. The xanthine precursors dissolved in DMF (1 mg / 0.4 ml-1 ml) were methylated in the presence of 10 mg potassium carbonate at 70°C for 10 minutes. Afterwards the reaction mixture was neutralized with 25 μl 4M HCl, then was analysed by analytical HPLC on a LiChrosphere RP 18 (250-4) column (Merck Co., Darmstadt), using different aqueous acetonitrile mobile phase systems. The flow rate of the eluent was 1 ml/min, and the peaks were detected using UV detector (Waters Co., USA) at 290 nm, coupled in series with a radioactivity detector.

Results

The [^{11}C]methylation reaction proceeds with high radiochemical yield in the presence of potassium carbonate in 10 minutes at 70 °C. Under these conditions only the desired N-[^{11}C]-methylation of the xanthine derivatives was observed. The components of the [^{11}C]-methylation reaction mixtures were identified by their Rt values, which were calibrated by the Rt value of the corresponding inactive standard taken prior to the measurement.

The process can be conducted with good (35-93%) radiochemical yield, calculated based on [^{11}C]methyl iodide. The specific activity was 1.85-5.55 GBq/μmol (50-150 mCi/μmol).

Synthesis of [^{11}C]CSC

Radiosynthesis of [^{11}C]CSC was carried out by the before mentioned method. (*E*)-8-(3-Chlorostyryl)-1,3,-dimethylxanthine in DMF (1mg/0.4 ml) was methylated in the presence of potassium carbonate or caesium carbonate at 60^{0}C for 10 minutes. Afterwards the reaction mixture was neutralised with 25 ml 4mol/l HCl, and the solution was purified by preparative HPLC on a LiChrosphere RP 18 (250-10) column (Merck Co., Darmstadt), with CH_3CN:water 7:3 at a flow rate 4 ml/min and detected at 290 nm by UV detector (Waters Co., USA), coupled in series with a radioactivity detector. (*E*)-8-(3-chlorostyryl)-1,3,-dimethylxanthine and [11C]CSC were eluted in the range of 5.5-6.5min and 8.5-9.5 min, respectively. After vacuum distillation the end product was dissolved in 0.2 ml DMSO and diluted with 10% DMSO in isotonic saline which contains 10% ethanol (pH=5).

The final solution was identified by analytical HPLC system on a LiChrosphere RP 18 (250-4) column (Merck Co., Darmstadt), with 70% acetonitrile as eluent at a flow rate 1 ml/min detected on UV detector (Waters Co., USA) at 290 nm, coupled in series with a radioactivity detector. The retention times of (*E*)-8-(3-chlorostyryl)-1,3,-dimethylxanthine and [^{11}C]CSC were 3.0-3.5 and 4.9-5.6 min, respectively.

The decay-corrected radiochemical yield was 30% based on [^{11}C]methyl iodide. The best specific activity was 9.1 TBq/μmol (246 mCi/μmol). No precursor was observed in the end product, and radiochemical purity was >99%. The *E*-to-*Z* isomerization of [^{11}C]CSC in the final solution was not observed.

Pharmacological characterisation of the prepared caffeines

Contractility assays have been done with CSC, 3,5-DMSC, 3,4,5-TMSC and ISC by using ZM 241385 as standard non-xanthine A_{2A} receptor antagonist. The compounds were characterised, by the pA_2 Schild plot slope parameter, calculated using electrically driven guinea pig atrial myocardium (reported to possess both A_1 receptors), guinea pig pulmonary artery (reported to possess A_1 and A_{2B} receptors) and rat pulmonary artery (reported to possess A_{2A} receptors) tissues.

CSC showed a remarkable A_{2A} (pA_2=6.49), especially A_{2A}/A_{2B} (56) selectivity. Although 3,5-DMSC (6.58) and 3,4,5-TMSC (6.59) are A_{2A} selective compounds, their selectivity did not achieved the above effect of the CSC. A significant A_{2A}/A_1 (468) selectivity was showed by the ISC (7,67), which pA_2 value showed nearly the same A_{2A} affinity like the nowadays known strongest A_{2A} inhibitor: ZM compound (pA_2=7,91).

Biological assay of [^{11}C]CSC

Competition experiments on DDT1 MF2 cells

Prepared, transformed (1 million/l) DDT1 MF2 muscular tissue cell lines were incubated at 37°C for 20 minutes with 20 μCi/ml [^{11}C]CSC. For displacement assays a 10 minute preincubation was performed with inactive CSC or ZM 241385 (known competitive antagonist). The cells were separated (SKATRON Cell Harvester, 11019), and the [^{11}C]CSC accumulation was registered by a calibrated gamma-counter (Canberra Packard). The radioactivity concentrations were expressed as cps (count per second)/ 1 million cell.

Autoradiography

In the autoradiography (ARG) studies storage phosphor screens (Molecular Dynamics) were used in combination with the PhosphorImager® scanner. The average thickness of the fresh brain and heart sections was 500 μm. The exposure

times fell between 15-60 minutes. The resolution of the scanned ARG images was 200 μm/pixel.

For anatomic correspondence a transparency scanner (HP ScanJet 4c/T) was used to obtain the colour images of the sections. ARG and TS images were registered to fuse functional and anatomical information.

Biodistribution

Biodistribution of [^{11}C]CSC was studied on Swiss mice after i.v. injection of the ligand. Male Swiss mice were injected by [^{11}C]CSC [120-200 μCi (4.44- 7.4 MBq) / 1.2-8 nmol] intravenously as a bolus. The animals were killed by cervical dislocation, dissected and various organs/tissues were removed at 10, 20, 40 and 60 minutes after the injection.

The radioactivity of the samples was measured, after precise weighting, by a calibrated gamma-counter. Plasma was obtained from blood by centrifugation (2min/3000g). Uptake values were expressed as Differential Absorption Ratios (DAR = cpm recovered/g tissue/cpm injected/body weight).

PET scanning

Rabbits were anaesthetised with i.p. urethane (0.5g / kg) and alpha-chloralose (50 mg/kg). One femoral artery and one femoral vein were cannulated. Serial arterial blood sampling was performed in all cases to allow kinetic analyses of [^{11}C]CSC accumulation.

Dynamic PET scans were carried out using GE 4096 whole body PET camera. The animals got 1-2 mCi/30-60 nmol [^{11}C]CSC in 2 ml physiologic salt solution intravenously as a 20-30 sec bolus. Transmission scans have been used to correct for tissue attenuation.

Based on the results of PET investigations and performing defined mathematical transformation we have modelled the kinetics of the [^{11}C]CSC accumulation. The method demands the follow up of the [^{11}C]CSC concentration in the blood by taking blood samples and acquiring a dynamic set of images. These data allows to calculate the time course of blood activity (blood curve) and tissue activity within a defined region of interest (ROI TACT) as the two input functions of the kinetic tracer model calculation. The calculation results in a set of numerical data for kinetic constants K_1, k_2, k_3,…k_6 which belong to the chosen ROI(s). For our calculations four compartment method [Landaw E. M., 1984], and "MATLAB" mathematical program was used.

Results

Competition experiments with the unlabeled ligand proved [^{11}C]CSC to bind specifically to the appropriate receptor. The measured bound [^{11}C]CSC decreased in the presence of inactive CSC or ZM241385.

Foci of high CSC accumulation were clearly localised on the brain autoradiograms in several cases, with 15 minute post injection slicing, and were in accord with previous findings on specific spatial distribution of A_{2a} adenosine receptors in the CNS.

The results of tissue distribution of [^{11}C]CSC in mice shows, that the highest uptake at 10 min after the injection was found in the lung followed by the liver, kidney, heart and brain. The kinetics of the accumulation of radioactive CSC was different in the various tissues. The very high uptake in the mice lung observed at 10 minutes after the injection gradually decreased in time. The brain uptake was almost the same as the heart uptake, and both showed a gradual decrease at later times (20, 40, and 60 min after the injection). The lowest radioactivity level was detected in the blood.

Dynamic PET studies on rabbits showed a fast brain uptake of CSC, reaching a maximum in less than 2 minutes.

Based on the four compartment method, using the blood curve and brain TACT curve obtained by dynamic PET images a set of numerical data for kinetic constants of the transport and binding of CSC in brain have been calculated.

Conclusion

Ten ^{11}C-labelled caffeine derivatives were prepared using [^{11}C]methyl iodide. Two new compounds, namely *E*)-8-(3-iodostyryl)-1,3,7-trimethylxanthine (ISC) and (*E*)-8-(3-iodostyryl)-1,3-dimethylxanthine (ISX) were prepared. The [^{11}C]methylations can be conducted with high radiochemical yields, using an automated synthesis procedure. The radiochemical synthesis was optimised by choosing the best catalyst, and the adequate reaction time and temperature. New, automated separation (preparative HPLC) and identification (analytical HPLC) methods were developed.

The newly prepared ISC adenosine antagonist seems to posses remarkable A_{2A}/A_1 selectivity.

The described [^{11}C]-caffeines seem to be potential subjects of future biological characterisation in order to evaluate their affinity and sensitivity for adenosine receptors of type A_{2a} in various tissues using PET-technique.

The ^{11}C-labeled selective adenosine A_{2a} antagonist [^{11}C]CSC was prepared using the above method. Based on the results of the animal studies, the [^{11}C]CSC seems to be a very promising radiopharmacone for mapping adenosine receptors of type A_{2a} in various tissues, especially in the CNS. It is also suitable to perform investigations concerning time-dependent receptor expression changes (up- and down-regulation).

THIS THESIS IS BASED ON THE FOLLOWING PAPERS

1. **Boros I**., Lengyel Zs., Balkay L., Horváth G., Szentmiklósi A.J., Fekete I., Márián T. Az A_{2A}-adenozin-receptor-eloszlás in vivo tanulmányozása ^{11}C-CSC-radioligandummal. Orvosi Hetilap. 143/21, Suppl. 2, 89-91,2002.

2. **Boros I.,** Horváth G., Lehel S., Márián T., Kovács Z., Szentmiklósi J., Tóth G., Trón L. [^{11}C]-labelling of some caffeine derivatives for mapping Adenosine A_{2A} receptors by PET technique. J. Radioanalytical and Nuclear Chemistry, 242, 309-313, 1999. Impact factor: 0.605

3. Márián T., **Boros I.,** Lengyel Z., Balkay L., Horvath G., Emri M., Sarkadi E., Szentmiklósi J., Fekete I., Tron L.: Preparation and evalution of [^{11}C]CSC as a possible tracer mapping adenosine A2A receptors by PET. Appl. Rad. Isot. 50/5, 887-893, 1999. Impact factor: 0.716

4. Lengyel, Zs**., Boros, I.,** Márián, T., Sarkadi, E., Horváth, G., Kovács, Z., Trón,L.: Possible use of ^{11}C-labelled 8-(3-chlorostyryl) caffein (CSC) mapping A_{2A} adenosine receptors in the CNS and myocardium. In Radioactive Isotopes in Clinical Medicine and Research XXIII . Eds: Bergmann H, Köhn H, Sinzinger H. Birkhauser Verlag 387-391, 1999.

5. Balkay L, Molnár T, **Boros I.,** Lehel Sz, Galambos T: Quantification of FDG uptake using kinetics models. In Positron Emission Tomography: A Critical Assesment of Recent Trends, edited by Gulyás, B. and Müller-Gärtner, H. W., Kluwer Academic Publisher, Dordrecht. 153-162, 1998.

6. Trón L, Ésik O, Borbély K, …Boros I, …: Első hazai tapasztalatok pozitron emissziós tomográfiás (PET) vizsgálatokkal. Orvosi Hetilap, 138 (5), 259-271, 1997.

Publications included partly in this thesys

1. Márián T., Rubovszky B., Szentmiklósi A. J., Tron L., Balkay L., **Boros I.,** Székely A., Krasznai Z.: A_1 and A_2 adenosine receptor activation inversely modulates potassium currents and membrane potential in DDT1 MF-2 smooth muscle cells. 2002. Jpn. J. Pharmacol. 89, 366-372. 2002. Impact factor: 1.21

2. Lehel Sz., Horváth G., **Boros I.,** Trón L.: Investigation for the nucleophilic substitution reaction of [^{18}F]fluoride ion on the series of N^6-benzoyl-2',3'-isopropylidine-adenosine-5'-sulfonates. J. Radioanalytical and Nuclear Chemistry, 251, 309-312, 2002. Impact factor: 0.605

3. Mikecz P., Tóth Gy., Horváth G., Lehel S., Kovács Z., Priboczki É., **Boros I.,** Miklovicz T., Márián T., Radiogyógyszerek előállítása pozitronemissziós tomográfiai vizsgálatokhoz, Pozitronemissziós tomográfia Magyarországon: Eredmények a klinikumban és a kutatásban. Orvosi Hetilap. 143/21, Suppl. 2, 12-14, 2002.

4. Gulyás B., Csiba L., …**Boros I.,** …: Egyszeri vinpocetin (cavinton) infúzió agyi anyagcserére gyakorolt hatásának vizsgálata pozitron emissziós tomográfiával (PET) krónikus stroke betegeken. Orvosi Hetilap, 142(9), 443-447, 2001.

5. Lehel Sz., Horváth G., **Boros I.,** Mikecz P., Márián T., Szentmiklósi J., Trón L.: Synthesis of 5-N-(2-[^{18}F]Fluoroethyl)-carboxamidoadenosine: a promising tracer for investigation of adenosine receptor system by PET technique. J. Labelled Compounds and Radiopharmaceuticals, 43, 807-815, 2000. Impact factor: 0.941

6. Lehel Sz., Horváth G., **Boros I.,** Mikecz P., Márián T., Trón L., Production of 5'-deoxy-5'-[^{18}F]fluoro-adenosine in the nucleophylic radiofluoration reactions of 5'-deoxy-5'-haloadenosine derivatives. J. Radioanalytical and Nuclear Chemistry, 245, 399-401, 2000. Impact factor: 0.605

7. Szakáll S., **Boros I.,** Balkay L., Emri M., Fekete I., Kerenyi L., Lehel S., Márián T., Molnar T., Varga J., Bereczki D., Csiba L., Gulyas B.: Cerebral effects of a single dose of intravenous vinpocetine in chronic stroke patients: a PET study. J. Neuroimaging, 8(4): 197-204, 1998. Impact factor: 1.044

8. Trón L., Balkay L., **Boros I.,** Emri M., Márián T., Molnár T., Tóth Gy., Gulyás B.: Positron Emission Tomography (PET)- One of the most advanced imaging techniques, Neurobiology 3 (2), pp. 205-206, 1995.

Presentations, posters

1. Márián T., Lehel S., Balkay L., **Boros I.,** Horváth G., Fekete I., Szentmiklósi J., Trón L., Adenozin-receptorok és PET-izotóppal jelölt ligandumjaik kölcsönhatásának in vivo és in vitro vizsgálata. Ötéves a Magyar PET Program, Tudományos Ülés, 1999. szeptember 22., Debrecen.

2. Márián T., **Boros I.,** Lengyel Zs., Szentmiklósi J., Fekete I., Balkay L., In vivo és in vitro vizsgálatok PET radiofarmakonokkal. DOTE PET Centrum Tudományos Ülés 1999. február 22., Debrecen.

3. Szentmiklósi J., **Boros I.,** Horváth G., Márián T,: Halogénezett sztirilkoffein származékok, mint potenciális PET tracerek: az A2a adenozin receptor szelektivitás farmakológiai elemzése. DOTE PET Centrum, Tudományos Ülés 1999. február 22., Debrecen.

4. Lengyel Zs., **Boros I.,** Márián T., Sarkadi E., Horváth G., Kovács Z., Trón L., Possible use of ^{11}C-labelled 8-(3-chlorostyryl) caffein (CSC) mapping A_{2A} adenosine receptors in the CNS and myocardium. Eur. J. Nucl Med. 1998, 24(1), S20 (Radioactive Isotopes in Clinical Medicine and Research, 23rd Int.Symposium, Badgestein, Austria, Januar 13-16, 1998.)

5. Lengyel Zs., **Boros I.,** Márián T., Sarkadi É., Szentmiklósi J., Horváth G., Trón L. (1997): In vivo and in vitro investigations with ^{11}C-labeled 8-(3-chlorostyryl)caffeine (CSC) as a possible PET tracer for mapping A2A adenosine

receptors in the CNS and myocardium. Fifth Symposium of International Society for Neuroimaging in Psychiatry, Groningen, November 27-29, 1997, pp. 29.

6. Horváth G., Márián T., **Boros I.,** Lengyel Zs., Lehel S., Kovács Z., Szentmiklósi J., Trón L., [^{11}C]-labeled 8-(3-chlorostyryl)caffeine (CSC) as a possible PET tracer for mapping A2A adenosine receptors in the CNS and myocardium. Conference on Achievements and Prospects of new Radiotracers, 13-14 November, 1997, Jülich, Germany, pp: 17-18.

7. **Boros I.,** Horváth G., Lehel Sz., Kovács Z., Márián T., Sarkadi L-né., Trón L., A_2 szelektív adenozin receptorligandum antagonisták elõállítása a DOTE PET Centrumban. Konferenciaposzter és elõadás, MONT X. Kongresszus, Bükfürdő, 1997 szeptember, Magyar Radiológia Supplement 1997(1), 12.

8. **Boros I.,** Horváth G., Lehel S., Trón L., Radiokémiai fejlesztési programok a debreceni PET Centrumban, Konferenciaposzter és elõadás , MONT X. Kongresszus, Bükfürdő, 1997 szeptember, Magyar Radiológia, 1997(1) 12.

9. Lehel S., Horváth G., **Boros I.,** Trón L., Adenozin receptorok vizsgálatára alkalmas ^{18}F-jelzett vegyületek elõállítása, Konferenciaposzter és elõadás, MONT X. Kongresszus, Bükfürdő, 1997 szeptember, Magyar Radiológia, 1997(1), 12.

10. Balkay L., Emri M., Molnár T., Márián T., **Boros I.,** Lehel Sz., Trón L. (1997) Kvantitatív szöveti glukóz anyagcsere térképek elõállítása PET mérések tracer kinetikai analízisével. Poszter, Sejt- és fejlõdésbiológiai napok, Debrecen, 1997. jan.19-22.pp. 23.

11. **Boros I.,** Lehel S., Tóth Gy., Trón L. [^{18}F]Fluoride production at the Debrecen PET Centre, conference poster, XXI-st World Conference of the International Society for Fluoride Research, Budapest, Hungary, August 1996 (conference book p54).

12. Lehel S., **Boros I.,** Tóth Gy., Trón L. 2,3-O-isopropylidine 5-triflic-adenosine: a promising precursor for the synthesis of [^{18}F]-labelled adenosine derivatives, conference poster, XXI-st World Conference of the International Society for Fluoride Research, Budapest, Hungary, August 1996 (conference book p53).

13. Illés A., Márián T., **Boros I.**: PET studies on Hodgkin's lymphoma patients "PET in Eastern and Central Europe", International Workshop, Debrecen, Hungary, 23-25 May 1996.