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Facile One-Pot Strategy For Radioprepreparationof Radioiodinated Phenylpiracetam As A New Highly Selective Radiotracer For Brainimaging

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In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract
The drug, phenylpiracetam, (PHPI, Iodophenylpiracetam ([131 I]-PHPI)) can prevent retrograde amnesia, and has anticonvulsant properties in models of animal was labeled using [I-131]with Chloramine-T(Ch-T) as an oxidizing agent to give a radiochemical yield of 98%. Many operators such as the amount of oxidizing agent, amount of substrate, pH, and reaction time, was systematically studied giving optimum conversion (98%) was obtained. Biodistribution studies indicate the[131I]PHPI tracer focuses on the target organ (brain) with a high percentage that is appropriate to use as a novel tracer for brain imaging. A labeled compound of [131I]-PHPImay be considered a highly selective radiotracer for brain tumor imaging compared withcommercially available radiotracers [99mTc] ECD and [99mTc] HMPAO.
Keywords:Phenylpiracetam, I-131, Labeling, Brain imaging, Biodistribution studies

1. Introduction

Brain imaging techniques provide the ability to noninvasively map the structure and functions of the brain. This is achieved either by directly measuring the currents and magnetic fields produced by neural activity, by injecting radioisotope agents to outline regions through emitted radiation or by measuring tissue-specific responses to an externally applied energy source such as a magnetic field. The obtained signals provide identifying information about the structures and physiological activities of the brain lending answers to questions about structural integrity, relevant particularly in clinical applications, as well as relating brain function to human cognition and behavior.

Many studies was assessed many radiotracers as brain imaging radiotracers [1-10]. Here it is worth mentioning the critical point in the brain imaging process, which most previous radiotracers suffer from, which is the small uptake value, as well as the failure of the sequence to continue inside the brain for an appropriate period.Accordingly, there has been interest in studying a drug that can overcome these aforementioned consequences, such as the drug phenylpiracetam [11-15].Since it has been proven that this drug, phenylpiristam(Figure 1), has the ability to reverse the inhibitory effects of the benzodiazepine diazepam, and it also increases the worker's behavior, prevents post-rotational nystagmus, and can also prevent retrograde amnesia.This has previously been studied in Wistar rats with gravity-induced cerebral ischemia.This drug also helped favor the restoration of local cerebral flow when the carotid arteries were blocked [16-20].As a result, the present work focuses on the labeling of phenylpiracetam(PHPI) with the imaging radionuclide, I-131 for the preparation of [^{131}I]-PHPI radiotracer as a possible diagnostic tool, testing its accumulation in the brain of Swiss Albino mice weighing 35 to 40 g.

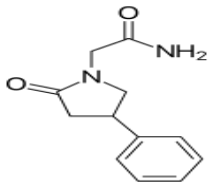

Fig.1.The structure of phenylpiracetam

The biological distribution was thoroughly calculated and the parameters affecting the labelled compound, [^{131}I]-PHPI radiotracer labeling yield were discussed totally [20-26]. By addressing the drawbacks of the available radiopharmaceuticals and

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paving the path for more effective diagnostic tools, this study contributes to the exploration of the radiotracer, [^{131}I]-PHPIas a potential breakthrough in brain imaging.

2. Materials and Methods

2.1. Solvents and Chemicals

Phenylpiracetam was purchased from MedChemExpress, USA and All the rest chemicals were analytical reagents grade purchased from Sigma-Aldrich Company. Thin layer chromatography (TLC) aluminum sheets (20 × 20 cm) SG-60 F254 were supplied by Merck. Whatman paper number (PC) 1, Whatman International Ltd, Maidstone, Kent, UK. All chemicals were of analytical or clinical grade and were used directly without further purification unless otherwise stated. Radioisotopes manufacturing facility (RPF) as an apart of Atomic Energy Authority, Inshas, Egypt, donated sodium [131I] iodide radiating (3.8 GBq/mL) alkalinized and dispensed in 0.05 M NaOH, pH 8 to 11 for radiolabeling. All additional chemicals and solvents used in analysis came from Merck Co.

2.2. Instrumentation and analysis

The radioactivity was measured by using a well-type detector NaI scintillation counter device with Scalar Ratemeter SR7 manufactured by Nuclear Enterprises Ltd., USA. The radioactivity was detected totally in a well-type NaI(Tl) – radiation emission counter (BLC-20, BUCK Scientific). Gel filtration using Sephadex 25-G medium was used to determine radiochemical yield. Glass column (0.5 × 31 cm) was packed with swollen gel (3 g of Sephadex and 30 mL normal saline heated at 90°C in a water bath for 1 h). The matrix was washed with 10 folds column volume using 0.9 N NaCl. Five hundred microliter of the reaction mixture was added to the top of the column, 0.9 % NaCl was used as eluant and the flow rate was 0.5 mL/2 min. Fractions of 0.5 mL were collected and counted using NaI (Tl) γ-ray scintillation counter.

2.3. PHPI radiolabeling

The drug, PHPI was formulated in ethanol (1 mg/mL) and was added in a variety of 1 ml Eppendorf tubes at various concentrations. Different concentrations of ChTin ethanol (1 mg/mL) solution were applied to the above-mentioned media. Throughout 30 minutes, the reaction mixture was agitated at 37 degrees Celsius in a water bath with a thermostat. The reaction was terminated by adding 300 µg NaMbiS with a concentration of 90 mg of sodium metabisulphite per ml of pH 6, to neutralize the excess iodine [22]. Ideal conditions resulted in a specific radioactivity of 18x 105 GBq/mmol for [^{131}I]-NaI(7.5x10 3 GBq in 0.1% NaOH). The radiochemical conversion to [^{131}I]-IodooPHPI was measured utilizing aluminum-coated silica gel GF254 plates. The reaction mixture, in a volume of 5 µL (1.86 MBq), was spotted over the plate edge from a height of roughly 1 cm. In a saturated elution chamber with chloroform: ethanol (8:2, v/v) as mobile phase, the plate was eluted to 75% of its size. The strips were detached, air-dried, and then cut off into 1cm pieces before being estimated for radioactivity emission with an SR.7 -counter. Based on GC analysis, it was found that [131I]-IodooPHPI had a purity of 99% [26-50].

2.4. Animal studies

Animal experiments were performed in compliance with the guideline established by the Animal ethics committee, Labeled Compounds Department, Egyptian Atomic Energy Authority. It was also in agreement with the rules of British Animal Protection (BAP).Swiss albino male mice approximately 8-10 weeks of age and weighing 35-45g, were obtained from Animal House, Biology Department, EAEA, Cairo, Egypt. The animals were kept upping at consistent nourishing conditions all through the trial time and kept at room temperature (22 ± 2 °C) with a 12 h on/off light schedule. The mice were bred in a cage with a free diet and water [51-70].

2.5. Physicochemical estimation

2.5.1. Biodistribution and animal studies

About 30 Swiss albino mice ranging in weight from 35 to 45 g were utilized for the biodistribution analysis. Tail vein injections of 0.2 mL of [131I]-IodooPHPIat physiological pH was given to 6 groups of normal mice (n=5). At 5, 15, 30, 60, 120, and 180 minutesafterinjections (p.i.), animals were sacrificed to measure organ distribution during the study. Isolating and measuring [131I]-IodooPHPI concentrations across many organs against a labeled substrate which served as a reference standard. Additionally, samples of blood, bone, as well as muscle were taken and estimated. A standard deviation (SD) for the mean value of the percentage of the supplied dose per gram was determined. It was calculated that muscle made up 40% of a person's body weight, whereas bone made up 10%, and blood made up 7% [71-80]. Experiments were conducted while corrections were used for background radiation and emission decays. The findings were analyzed with a one-way ANOVA test. We used the mean standard deviation to summarize the data, and we regarded a P value of lower than 0.05 to be statistically substantial.

6. Determination of stability

By combining 1.8 mL of normal rat fresh serum with the addition of 0.2 mL of a pure radiotracer, [^{131}I]-IodooPHPIby volume (v/v) [0.15 x 10-3 GBq], the serum stability of [131I]-IodooPHPIwas determined and stored at room temperature. [^{131}I]-IodooPHPI{5.0 µL(3.0 x 103 GBq)} stability in normal saline was also evaluated. The radiotracer and [^{131}I]-IodooPHPI, were exposed to thin-layer chromatography (TLC) for stability testing before being counted in a well-type – radiation emission scintillation counter.

2.6. Statistical analysis

Data were analyzed using Prism 5.03 (GraphPad, San Diego, CA, USA) and expressed as means ± standard error. Comparisons between groups were analyzed by one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Evaluation of radiochemical yield by TLC, paper electrophoresis, and gel filtration (GF)

The percent (%) on TLC at RF 0.9 to 1.0 as well as Rf 0.0 to 0.1 was used to calculate the radiolabeling yield of [131I]-IodooPHPI to free [131I] iodide. With the help of a NaI (Tl) -ray scintillation counter, we were able to determine that a maximum conversion of 98.0% had been achieved. Also, paper electrophoresis results, [^{131}I]-IodooPHPIremained at 0 cm from the spotting point (neutral labeled compound). free [^{131}I]-iodide moved towards the anode at a 11 cm distance from the spotting point. An optimum conversion of 98% was achieved.Gel filtration of samples from the reaction mixture resulted in two peaks. First, [131I]-IodooPHPI eluted at fraction 14 then the free [131I]-iodide at fraction 25 while the rest species was retained on the column is shown in Fig.2. The percentage of the labeling yield of [131I]-IodooPHPI was determined as the percent ratio of [131I]-IodooPHPI [81-100].

3.2. Reaction optimization

The reaction mixture's pH, oxidizing agent concentration, temperature, stability, as well as substrate concentration were all optimized. If the amount of substrate is increased to 100 µg while keeping all other reaction parameters constant, the maximal conversion to [131I]-IodooPHPIis 98.0%. (Figures 3A-3D). Maximum conversion to [131I]-IodooPHPIrequires careful regulation of the reaction mixture's pH.pH6 was found to be ideal (conversion of 98.0%), which may be indicative of the stability of [131I]-IodooPHPI. At very acidic or basic pH values, the optimum conversion to [131I]-IodooPHPIdecreased[100-110]. Additionally, the amount of oxidizing agent also has a significant role in the optimum transformation to [131I]-IodooPHPI. An increasing amount of oxidizing agent (Ch-T) to a ceiling of 100 µg proved to be optimal (98.0%) [100-112]. Increasing or decreasing the value of oxidizing content from 100 µg causes a decrease in the optimum conversion to [131I]-IodooPHPIwhile keeping the other parameters constant [100-111]. Studies tested the in-vitro radiochemical stability of [131I]-IodooPHPI[5 µL (3.80 MBq)] in 2 separate media. Purified [131I]-IodooPHPIcan maintain its 98.0% purity in a saline solution for as long as 24 hours. Following 12 hours, however, the purity in serum decreased to 89.0% [111-115] giving rise to enough time for hospital manipulation.

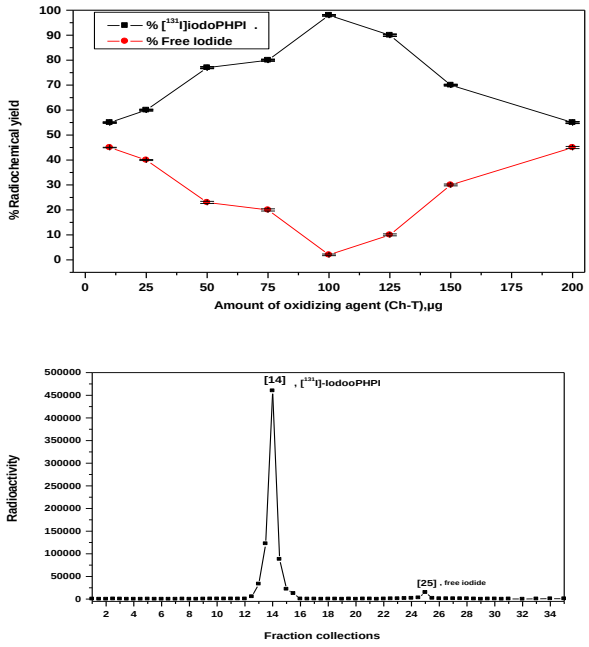

Fig. 2.Gel filtration radiochromatogram of [^{131}I]-IodooPHPI, n = 3.

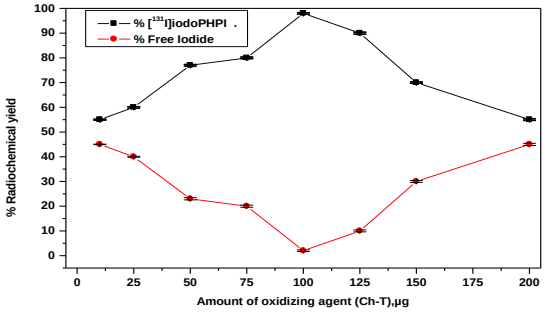

Fig. 3A Graph showing the effect of an oxidizing agent on the radiochemical yield under the reaction conditions: 10 µL (~3.7MBq) Na131I, 100 µg of PHPI, (x µg) of Ch-T, at pH 6; the reaction mixtures were kept at room temperature for 30 min.

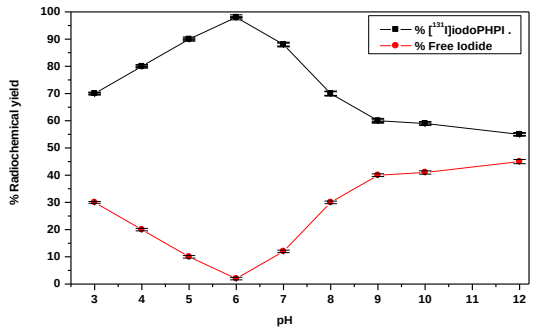

Fig. 3B Variation of the radiochemical yield of [^{131}I]iodoPHPIas a function of pH; reaction conditions: 10 µL (~3.7MBq) Na131I, 100 µg of PHPI, 100 µg of Ch-T, at different PHs; the reaction mixtures were kept at room temperature for 30 min.

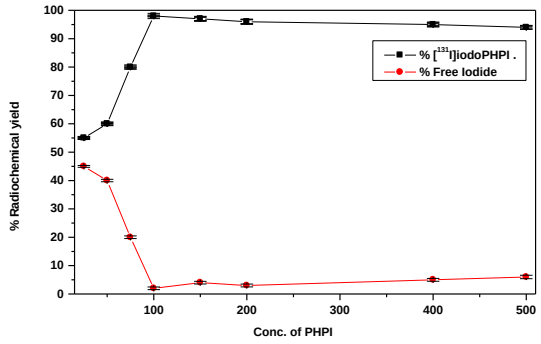

Fig. 3C Variation of the radiochemical yield of [131I]iodoPHPI as a function of PHPI amount; reaction conditions: 10 µL (~3.7 MBq) Na131I, (x µg) PHPIm, 100µg of Ch-T, at pH 6; the reaction mixtures were kept at room temperature for 30 min.

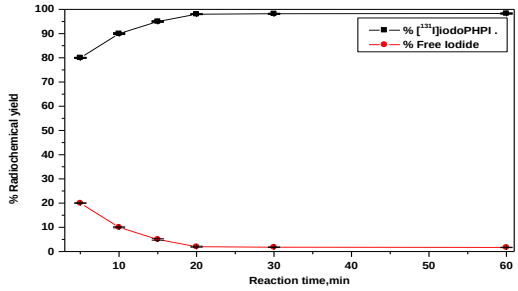

Fig. 3D Variation of the radiochemical yield of [131I]iodooPHPI as a function of reaction time; reaction conditions: 10 µL (~3.7 MBq) Na131I, 100 µg of PHPI, 100 µg of Ch-T, at pH 6; the reaction mixtures were kept at room temperature for different intervals of time.

3.3. Biodistribution study

The radiotracer, [131I]-IodooPHPI, was administered to mice and its bio-distribution in various organs, as well as fluids,are displayed in Table1. The standard unit of measurement for radioactivity is the standard deviation of the mean calculated injected dose for each gram per organ tissue (%ID/g organ)was used. [131I]-IodooPHPI stability ex vivo is demonstrated by its absorption by the thyroid gland [111-119]. Most organs get the radiotracer, [131I]-IodooPHPI, after 5 minutes post-injection[88-95]. After 5 minutes post-injection, brain uptake was 10.55ID/g%, and after 3 hours, it had declined to 2.11 ID/g%.Low thyroid uptake at all times indicates that the radiotracer [131I]-IodooPHPIcompound is stable in vivo to enzymatic de-iodiantion.The kidney uptake increased up to 17.67 ± 0.99% at 60 min p.i. and decreased to 3.77 ± 0.12% at 3hour. p.i. This indicated that the tracer is mainly excreted through urinary pathways.After 1 hour, the labelled compound[^{131}I]-IodooPHPIuptake notably decreased in most organs. The radiotracer, [^{131}I]-IodooPHPIshowed higher uptake in brain as target organ than recently developed agents such as[99mTc]-oxiracetam (5.1%),[99mTc]-tricabonyl oxiracetam (7.5%), [99mTc]-piracetam (6.0%), [99mTc]-Nitrido-Levetiracetam (4.5%), [125I]-Aniracetam (7.9%), [^{131}I]-Omberacetam (9.6%) at the same time P.I., 5 minutes. In addition, our radiotracer has uptake in brain more than two commercially radiotracer,[99mTc]-ECD and [99mTc]-HMPAO which have 4.7% and 2.25%respectively.Therefore,the labelled compound[^{131}I]-IodooPHPIcould be considered a better brain imaging agent. Our results indicate that the radiotracer,[^{131}I]-IodooPHPIhas a higher % ID/gram ±S.D value than these materials.

Table1. Biodistribution of [^{131}I]iodoPHPI in normal mice at different times

Organs	% I.D./g at different times post injection					
	5 min	15 min	30 min	60 min	120 min	180 min
Blood	9.11 ± 0.05	6.15 ± 0.14	4.19 ± 0.17	1.29 ± 0.16	1.11 ± 0.09	0.96 ± 0.00
Bone	1.19 ± 0.03	1.17 ± 0.06	1.13 ± 0.04	0.99 ± 0.00	0.97 ± 0.00	0.90 ± 0.00
Muscle	2.19 ± 0.03	2.27 ± 0.02	3.11 ± 0.03	3.11 ± 0.09	2.11 ± 0.08	1.19 ± 0.08
Brain	10.55± 0.20	7.13 ±0.36	6.12 ± 0.15	5.11 ± 0.18	2.99 ± 0.07	2.11 ± 0.02
Lungs	1.33 ± 0.12	1.29 ± 0.11	1.13 ± 0.09	1.12 ± 0.12	0.98 ± 0.00	0.97 ± 0.00
Heart	1.19 ±0.05	1.17 ±0.03	1.11 ±0.04	0.99 ± 0.00	0.95 ± 0.00	0.90 ± 0.00
Liver	3.17 ±0.19	4.17± 0.22	5.88 ±0.59	6.38± 0.23	5.11 ±0.27	2.16 ±0.49

Kidneys	5.22± 0.12	6.29 ±0.99	9.17 ±0.97	17.67 ± 0.99	12.42± 0.12	4.11± 0.18
Spleen	1.18 ±0.02	1.19 ± 0.04	1.22 ±0.08	0.99 ± 0.00	0.97± 0.00	0.92± 0.00
Intestine	2.11 ± 0.19	3.12 ± 0.14	4.12 ± 0.09	5.12± 0.97	2.99 ± 0.06	2.12 ± 0.07
Thyroid	1.13 ± 0.03	1.14± 0.07	1.11 ± 0.04	0.99±0.00	0.98±0.00	0.92±0.00

Mean±SD (mean of five experiments)

4. Conclusion

An optimized protocol for the synthesis of [131I]-IodooPHPIradiotracer in high yield has been developed at optimal conditions. From biodistribution studies, it was concluded that the [131I]-IodooPHPIradiotracer has a high uptake ratio in brain, target organ of 10.55 at 5 minand remains for up to 30minutes. Therefore, [131I]-IodooPHPIradiotracer could be considered a new radiopharmaceutical for brain imaging agents.

5. Conflict of interest statement

The authors declare that there is no conflictofinterest

6. References

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