ORIGINAL ARTICLE

PET imaging to assess fibroblast activation protein inhibitor biodistribution: A training program adapted to pharmacology education

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Abstract

In the process of pharmacology education, practical teaching is an important complement to theoretical teaching. These activities include the use of experimental animals to obtain certain pharmacological parameters or to help students understand certain classical concepts. However, the growing interest in laboratory animal welfare, the rapid development of pharmacology research and the challenges of cultivating innovative pharmacy talent create a need for innovative and flexible in vitro experiments for teaching purposes. Here, we report the application of positron emission tomography (PET) imaging of ^{18}F-labeled fibroblast activation protein inhibitor (^{18}F-FAPi) to practical pharmacology teaching, enabling dynamic visualization of the distribution and excretion process of FAPi in mice. Students can quantitatively analyze the distribution of FAPi in various tissues and organs without sacrificing the mice. Furthermore, the newly implemented method resulted in highly reproducible results and was generally appreciated by the students. Additionally, the application of PET imaging in pharmacokinetic teaching can not only greatly reduce the use of experimental animals but also need not sacrificing animals. Of note is that dynamic scanning data from this project can be used for online practical teaching during COVID-19 pandemic.

KEYWORDS
PET imaging, pharmacokinetics, pharmacy education

1 | INTRODUCTION

Pharmacology experiments accumulate the rich content of pharmacology theories and is crucial for future career development for students.[1] At present, pharmacology research methods are constantly being updated, while few apparent changes can be noted in pharmacology practical training for pharmacy students.[2] For example, in practical training on the histological distribution of drugs, mice are usually sacrificed for the collection of tissues or organs, and the drug concentrations in these specimens are then measured by high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC–MS).[3–5] This not only consumes large of experimental animals but also fails to provide an opportunity for visual observation of drug metabolism; thus, it is not conducive to the development of students' theoretical knowledge. Therefore, it is the goal of pharmacy teaching reform to replace animal experiments or reduce the use of laboratory animals with innovative experiments guided by the principle of the three R's (replacement, reduction, and refinement).[6–8]

Positron emission tomography (PET) or PET with computed tomography (PET/CT) is a sensitive and rapidly emerging technology for in vivo imaging after labeling compounds capable of participating in metabolic processes with positron-emitting radionuclides (e.g., 18F, etc.).[9,10] PET imaging is widely used to obtain crucial pharmacokinetic information non-invasively and has developed rapidly on drug development recent years.[11] The most distinctive features of PET imaging are that it can capture the process of drug distribution and excretion in animals or humans in a noninvasive and dynamic manner.[12–14] Introducing PET imaging into pharmacology teaching not only serves as an alternative to traditional animal experiments but also helps students to better understand drug pharmacokinetics and exposes them to new research tools, which can be helpful for their future career development.

FAP is highly expressed in activated fibroblasts, such as tumor-associated fibroblasts, glioma cells, rheumatoid arthritis synovial fibroblasts, etc.[15] Type II collagen-induced arthritis (CIA) in mice exhibits severe polyarthritis and can cause chronic, destructive joint damage, sharing many similarities with human rheumatoid arthritis. Therefore, it is widely used internationally for the study of RA pathogenesis and the development of innovative drugs. Previous studies have demonstrated that FAP expression in synovial tissues of CIA mice is higher than that of normal controls and that FAP expression is positively correlated with CIA arthritis scores. In recent years, there has been an explosion of research on PET imaging of isotopically labeled FAP inhibitors (FAPi) for the diagnostic evaluation of various diseases.[16] Therefore, we introduced ^{18}F-FAPi for CIA imaging into the teaching, in addition to wanting to let students observe the physiological metabolic process of FAPi, we also wanted to expand the depth of learning and let them understand the reasons for the targeted distribution of drugs.

In this innovative experiment, FAPi was radiolabeled with ^{18}F (^{18}F-FAPi) and dynamic scanning was performed in CIA mice after the tail vein injection of ^{18}F-FAPi. Students can not only observe the excretion process of ^{18}F-FAPi through dynamic scanning images but also quantitatively analyze the distribution of ^{18}F-FAPi in various tissues and organs. The experimental framework for teaching is shown in Figure 1.

2 | MATERIALS AND METHODS

2.1 | Collagen-induced arthritis model

The use of mice in this study was approved by Institutional Animal Care and Use Committee (IACUC) of Shandong Medicinal Biotechnology Centre. CIA was established and assessed as previously described.[17] Briefly, eight-week-old male DBA/1 J mice were purchased from Charles River (Beijing, China) and were acclimatized for 1 week after arrival. Then, the mice were immunized with 100 μg of bovine collagen type II (CII, Chondrex, Redmond, WA, USA) emulsified in an equal volume of complete Freund's adjuvant (CFA). At day 21, a booster immunization of CII emulsified in incomplete Freund's adjuvant (IFA, Chondrex) (2 mg/ml) was administered. The onset of arthritis occurred a few days after the booster immunization. The

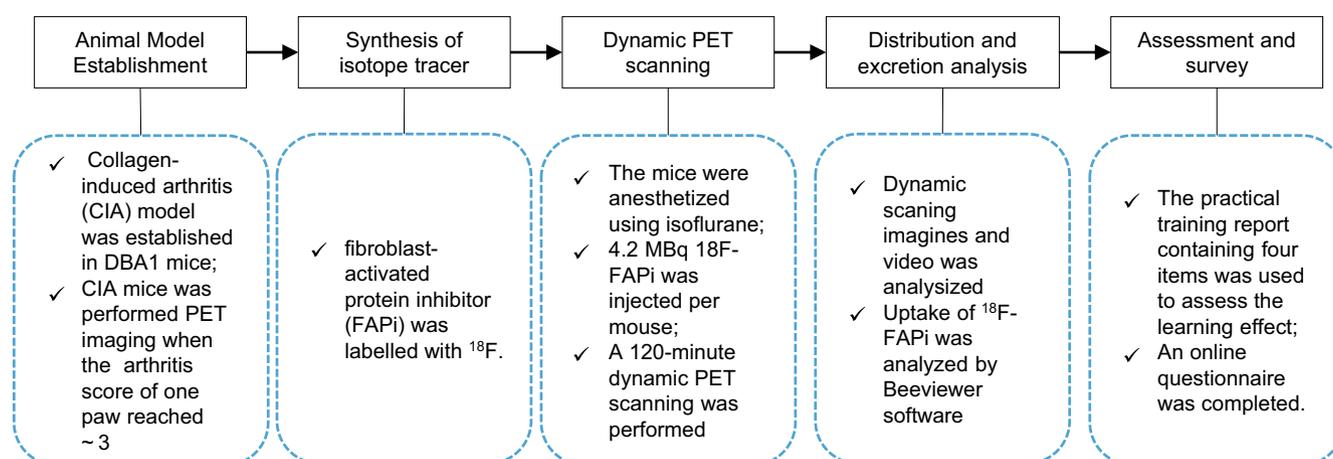| Animal Model Establishment	Synthesis of isotope tracer	Dynamic PET scanning	Distribution and excretion analysis	Assessment and survey
✓ Collagen-induced arthritis (CIA) model was established in DBA1 mice; ✓ CIA mice was performed PET imaging when the arthritis score of one paw reached ~ 3	✓ fibroblast-activated protein inhibitor (FAPi) was labelled with ^{18}F.	✓ The mice were anesthetized using isoflurane; ✓ 4.2 MBq 18F-FAPi was injected per mouse; ✓ A 120-minute dynamic PET scanning was performed	✓ Dynamic scaning imagines and video was analysized ✓ Uptake of ^{18}F-FAPi was analyzed by Beeviewer software	✓ The practical training report containing four items was used to assess the learning effect; ✓ An online questionnaire was completed.

FIGURE 1 Design and framework of the study

progression of arthritis in each paw was graded as follows: 0, no change; 1, mild swelling; 2, obvious joint swelling; and 3, severe joint swelling and ankylotic changes.[18]

2.2 | Radiotracer synthesis

[18]F-labeled FAPi (^{18}F-FAPi) was synthesized as described previously.[19] Briefly, 2–10 GBq ^{18}F in the form of fluoride ions, produced by a medical cyclotron (MINITRACE Cyclotron), was trapped on a quaternary methyl ammonium (QMA) cartridge and then eluted with 0.30 ml of saline. Six microliters of 10 mM $AlCl_3$ dissolved in sodium acetate buffer (pH 4, 0.1 M) and 200 µl of acetonitrile (Sigma–Aldrich) were mixed with NOTA-FAPi (100 µg, 130 nM) dissolved in sodium acetate buffer (60 µl, pH 4, 0.1 M). The mixture was heated at 110°C for 10 min, diluted with 10 ml of water and transferred to a C_{18} cartridge. The cartridge was washed twice with 10 ml of water, and the desired product was then eluted with 0.6 ml of ethanol. The product was dissolved in saline and filtered through a 0.22 µm Millipore filter into a sterile vial. The radiochemical purity of ^{18}F-FAPi was determined by radio-HPLC.

2.3 | Micro-PET imaging and data acquisition

Micro-PET/CT was performed as previously described.[19] Briefly, CIA mice were anesthetized using isoflurane and then injected with 4.2 MBq of ^{18}F-FAPi. A 120-min dynamic PET scanning in 5-min intervals was performed immediately. Micro-PET imaging was reconstructed using the standard ordered-subset expectation maximization method. The PET images were reconstructed using OSEM3D/MAP. Students participating in the project could use BeeViewer software to obtain standardized uptake values (SUVs) of the region of interest.

2.4 | Project implementation

The training program was conducted among 20 fourth-year undergraduate students and 20 first-year graduate students. Theoretical knowledge related to drug targets and pharmacokinetics was reviewed prior to the start of the program. Students established the CIA models under the guidance of their lead instructors, observed the mice daily after the second immunization to assess the arthritis score of the paws, and scored the degree of joint swelling. The students established the CIA model under the guidance of the project instructor and assessed the arthritis score every 2 days after the second immunization. When the arthritis score reached 3, the Micro-PET imaging was performed by the nuclear medicine physician and students in the project team. The nuclear medicine physician is responsible for the synthesis of ^{18}F-FAPi and the scanning of micro-PET/CT. The students conducted the injection of the tracer and the observation of the mouse status during the scanning. The acquired PET/CT images were sent to each student participating in the

project, followed by training by nuclear medicine physician on how to analyze the SUV value of each organ or tissue. Students graphed the analyzed SUV using GraphPad Prism (Version 8.0) software.

2.5 | Learning effect assessment

As an assessment, students participating in this training program were asked to write a practical training report containing four items. The first item was to analyze the uptake values of ^{18}F-FAPi in each organ and plot them by GraphPad prism software. The second item is to describe the excretion pathway of ^{18}F-FAPi. The third item is the explanations for the targeted distribution of ^{18}F-FAPi in inflamed joints. The fourth item is to study the advantages of pharmacokinetics by PET imaging. The practical training reports were scored by two project instructors. Each item was evaluated on a scale with five levels: very good, excellent, pass, poor, and very poor, which were assigned scores of 20–25, 15–19, 10–14, 5–9, and 0–4, respectively.

2.6 | Survey

Finally, an online questionnaire (in Chinese) was completed anonymously by the students who participated in the project. The survey consisted of five items, each of which could be answered on a 5-point Likert scale ("−2 = Strongly Disagree", "−1 = Rather Disagree", "0 = Neutral", "+1 = Rather Agree", and "+2 = Strongly Agree").[20] The items were designed to assess the students' overall perception regarding the main objectives of the practical training program: improving the understanding of drug metabolism processes, increasing interest in studying pharmacology, introducing students to research practices and data handling, and reducing the use of laboratory animals. The questionnaire took each student approximately 5 min to complete and was not mandatory for successful completion of the pharmacology course.

2.7 | Statistical analysis

GraphPad Prism was used for statistical analysis. Statistical analyses comparing two groups were performed using Student's t test (when the data were normally distributed) or the Mann–Whitney U test (when the data were not normally distributed). $p < .05$ was considered statistically significant (*$p < .05$).

3 | RESULTS

3.1 | Experimental protocol optimization

^{18}F-FAPi (Figure S1) was stably produced with high purity. To increase the uptake of ^{18}F-FAPi in the inflamed joints, we selected mice with relatively severe CIA (at least 1 paw with an arthritis score of 3) for PET imaging. Mice began to show signs of arthritis on day 25 after the

first immunization, and very pronounced signs of arthritis appeared by day 32, manifested by inflamed joints of the front and hind limbs. Then, dynamic scanning was performed in CIA mice for 120 min, and the representative images acquired were shown in Figure 2. The uptake of ^{18}F-FAPi in the blood, muscle, heart, liver, lung, and brain was low. However, high uptake ^{18}F-FAPi in the kidney, bladder, and inflamed joints could be clearly observed. The above results suggested the targeted distribution of ^{18}F-FAPi. Moreover, students can easily observe that ^{18}F-FAPi enters the bladder with urine from the kidney and enters the feces with bile secretion (Video 1).

3.2 | Student implementation

3.2.1 | Workshop setup and results

The following information about ^{18}F-FAPi distribution and excretion should be available to students before they analyze the images. First, ^{18}F-FAPi is specifically bound to FAP, which has been confirmed in previous studies. Second, the uptake of ^{18}F-FAPi is low in organs with low FAP expression, such as brain, blood, and lung. Conversely, high uptake of ^{18}F-FAPi occurs in inflamed joints due to high FAP expression in synovial cells. Third, ^{18}F-FAPi is excreted through urine and bile, so the distribution of ^{18}F-FAPi in kidney and bladder varies with the amount of urine. And the distribution in the intestine varies with bile secretion.

Then, students were instructed to analyze the uptake values of ^{18}F-FAPi by BeeViewer software. Through a short training session, the students were able to master the use of the software. Representative data analyzed by the students are shown in Figure 3. Each group can complete the data analysis.

3.2.2 | Learning effect assessment

To examine the effectiveness of the training, we assessed the practical training reports using the four-item assessment described above. Each item is scored on a scale of 0–25, with scores of 20–25, 15–19, 10–14, 5–9, and 0–4 indicating excellent, good, pass, poor, and very poor, respectively. As shown in Figure 4, both graduates and undergraduate students scored an average of over 20 for each item. Moreover, there was no difference in the scores of each assessment item in their practical training reports. This suggests that the students involved in this project have an understanding that PET imaging can be applied to pharmacokinetics.

3.3 | Survey

Participating students were invited to fill out an online survey, which was administered on a voluntary basis. Specifically, we assessed the extent to which students agreed with the following five items: (1) I have learned additional methods for studying pharmacokinetics through the training, (2) This training had contributed to my understanding of drug distribution and targeting characteristics, (3) This training had helped me develop my practical skills, (4) The application of PET imaging reduced the use of experimental animals, and (5) This training program contributed to my interest in learning pharmacology. Figure 5 summarizes the survey responses and shows that the students strongly agreed that the training program was beneficial; there was no significant difference between the scores given by the undergraduate and graduate groups.
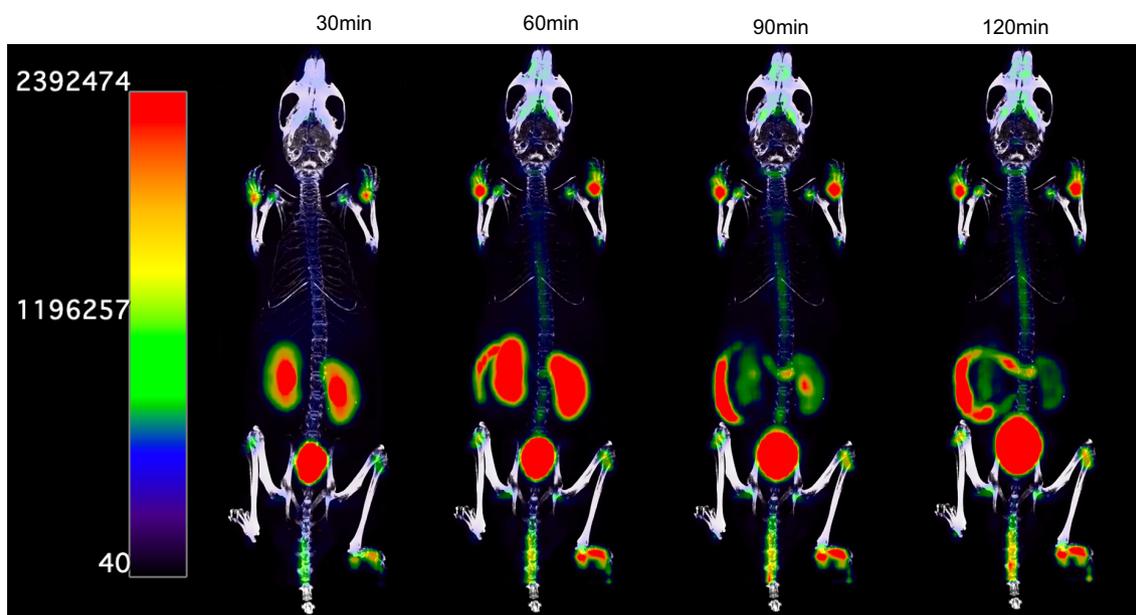

FIGURE 2 Representative images of dynamic scan. A 120 min dynamic scan was conducted after tail vein injection of ^{18}F-FAPi. Image acquisition was performed at 5-min intervals, and scans at the 30, 60, 90, and 120 min are shown.

FIGURE 3 Uptake values of ^{18}F-FAPi at different time points in different organs and tissues. Students analyze the distribution of FAP using BeeViewer software and perform graphical analysis. The results of one of the students are presented. Date was expressed as mean±SD.
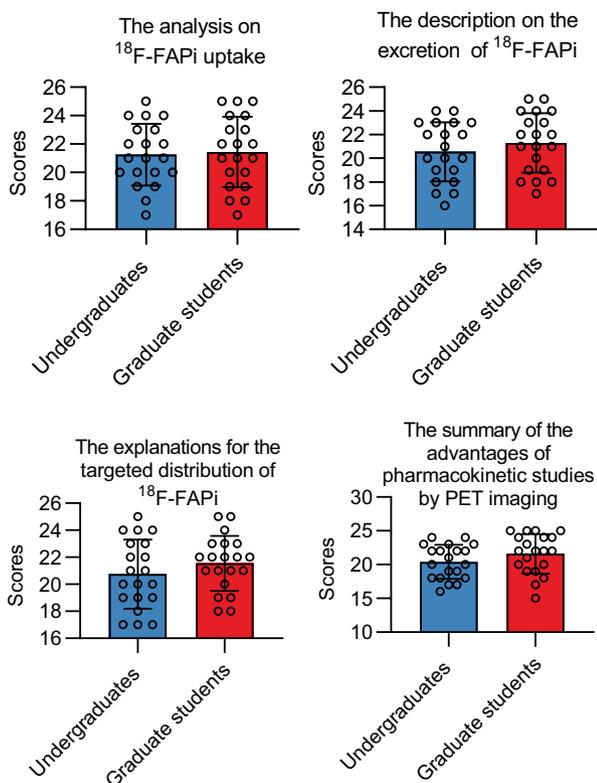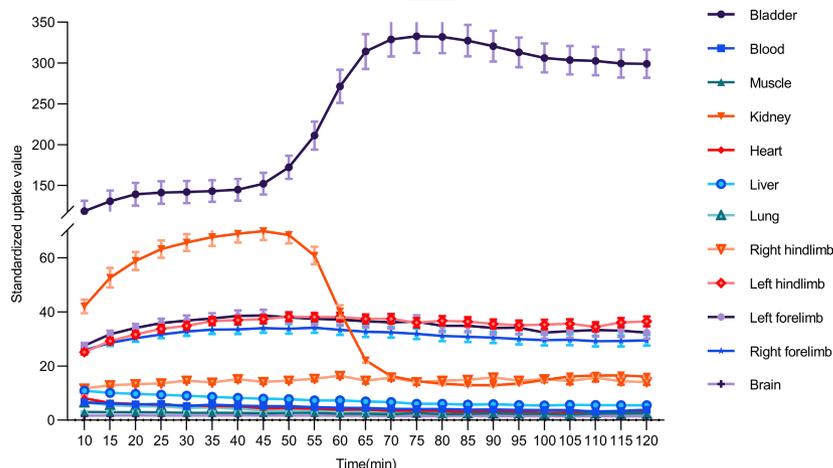

FIGURE 4 Learning Effect assessment. As an assessment, students participating in this training program were asked to write a practical training report containing four items. Each item was evaluated on a scale with five levels: very good, excellent, pass, poor, and very poor, which were assigned scores of 20–25, 15–19, 10–14, 5–9, and 0–4, respectively. Date was expressed as mean±SD.
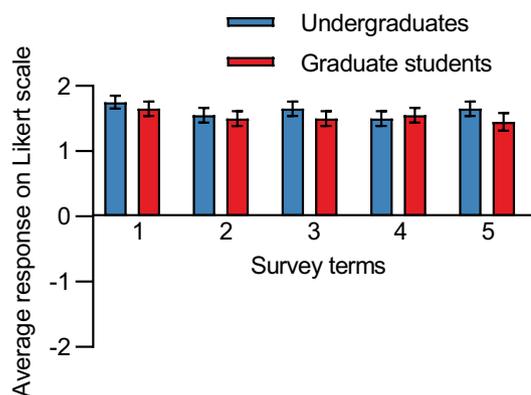

FIGURE 5 Learning outcomes were evaluated among students by means of a questionnaire. All responses were scored along a 5-point Likert scale (+2 = strongly agree; +1 = slightly agree; 0 = neutral; −1 = slightly disagree; −2 = strongly disagree).

4 | DISCUSSION

Guiding students to understand and master new pharmacological research techniques is a challenge for modern pharmacology teaching.[21] In the past, animal experiments were often used for the validation of classical theoretical knowledge.[22–24] One of these classical experiments is to isolate various organs at different time points after drug ingestion in mice and use HPLC or other methods to assay the drug content, thus allowing students to understand pharmacokinetic parameters and their implications. However, this type of experiment can easily lead to animal ethics issues because of the large number of animals that need to be used and the harm caused to the animals by the experimental methods.[25,26] Additionally, students can only calculate the distribution of the drug from the final data and cannot visualize the distribution of the drug using this method. These limitations are detrimental to the establishment of the knowledge structure and makes teaching more difficult.

PET has taken on an increasing role in disease diagnosis, especially in the early diagnosis of tumors and in monitoring the efficacy of tumor treatments. As radioisotope labeling tracers provide a noninvasive functional imaging modality, its application to exploratory studies of innovative drugs can expand the information available on pharmacokinetic equilibrium and the pathways and extent of excretion as well as target distribution and other important topics, reduce the possibility of underestimating potential risks in nonclinical trials, and improve the science of safety evaluation

for innovative drugs.[27,28] FAP is highly expressed in activated fibroblasts, such as tumor-associated fibroblasts, glioma cells, rheumatoid arthritis synovial fibroblasts, etc.[15] In recent years, there has been an explosion of research on PET imaging of isotopically labeled FAP inhibitors for the diagnostic evaluation of various diseases.[16] These studies have confirmed that isotope labeled FAPi specifically binds to FAP and that organs or tissues with high expression of FAP exhibit high uptake of FAPi.[29] In this manner, PET not only provides the pharmacokinetic parameters of FAPi but also demonstrates the expression profile of FAP in various diseases by using FAPi as a tracer.[30,31] If this method is introduced into teaching, it will expose students to more methods of pharmacokinetic studies, which is important for the training of future drug development talent.

In this training program, a laboratory instructor assists students in building a CIA model. Because it has been shown that FAP expression is significantly elevated in diseased joints in the CIA model,[32] PET imaging can capture significantly increased uptake of ^{18}F-FAPi in the inflamed joints. Studies have shown that the uptake values of nucleotide-labeled FAP antibodies or inhibitors correlate positively with the severity of arthritis.[32,33] To improve the reliability of the data obtained by the students, we optimized the experimental protocol by using mice with a more severe degree of joint pathogenesis, which would result in a more targeted distribution of ^{18}F-FAPi. Previous studies have demonstrated that ^{18}F-labeled FAP rapidly reaches equilibrium in the blood and that the concentration in the blood returns to a low value within a few minutes.[19] The concentration is very low in the liver and kidneys at 120 min, since the tracer is excreted mainly through bile and urine and reaches considerably high uptake in the bladder, gallbladder, and large intestine of mice as visualized by PET. In addition to these organs, it can be observed in PET images that inflamed joints also show high uptake, while disease-free joints still show low uptake because of the low expression of FAP. In this project, dynamic scanned images will be provided to the students so that they can visualize the distribution of the drug without needing to sacrifice the animal.

This training program was conducted among fourth year undergraduates and first year graduate students in pharmacy because they have enough theoretical knowledge and have received classical pharmacy practice training at the undergraduate level. Therefore, it was easier for these students to compare and analyze the benefits of this program applied to their teaching. Dynamic scans were performed in CIA mice for 120 min, with intervals of 5 min, beginning immediately after the injection of ^{18}F-FAPi. Using the software BeeViewer, students analyzed these images in three dimensions and obtained the uptake values of ^{18}F-FAPi for each organ by outlining the target areas. The practical training reports showed that these students could understand the project well and could acquire the necessary skills, and there was no significant difference in assessments scores between undergraduates and graduate students. Additionally, the questionnaire after the practical training showed that the students were very satisfied with the training and believed that it increased their interest in learning pharmacology.

However, there are also some limitations and concerns of this grogram. Not all teaching institutions are able to perform PET imaging, and the cost of PET imaging is still relatively high at this stage. It is possible that even if PET imaging could be performed, there would not be enough funds to implement the experiment. In fact, the process of nuclide labeling and imaging is not critical for students to understand pharmacokinetic parameters. It is the dynamic scan data, video and analysis of uptake values obtained through PET imaging that are most important for students to observe and understand the drug excretion and distribution process. Therefore, we believe that the data from this experiment can be reused when conducting this experimental training in the future. Teaching institutions can also conduct similar teaching activities by citing pictures and data from the literature, depending on the actual situation. In addition, the number of students involved in this experimental training is small, and when the number of students is larger, the instructor needs to do more preparation to implement this teaching activity, such as the reagents and materials needed for the experiment. In terms of safety, the use of ^{18}F is very common and has been widely used in scientific research and clinical practice, and its safety has been recognized.[34] In the future, we will continue to improve this experiment and will try to build a virtual simulation experiment platform. The virtual simulation experiment can be widely applied to institutions that cannot start PET imaging or for online teaching during the COVID-19 epidemic.[35]

In conclusion, we report here on the successful implementation of PET imaging for pharmacokinetics in the teaching. The experiment itself relied on materials available to the instructor, and the resulting dynamic scan images could be reused for subsequent teaching to provide reproducible results. This project provides a valuable and cost-effective simulated alternative to experiments that require the use of live animals for teaching, providing a suitable option to reduce the number of animal experiments associated with teaching. In this case, students learn about a technique commonly used in scientific research, and our instructional method could be replicated in teaching to benefit more pharmacy students. The implementation of this hands-on training program was a fruitful integration of scientific research and teaching activities, which was strongly appreciated by the students and furthered their understanding of key points of pharmacology. More importantly, PET-based pharmacokinetic studies are increasingly important in the modern drug development process; thus, our teaching method helps students become familiar with technological advances in the field, which will serve them very well later in their careers. In conclusion, this proposed hands-on training program provides instructors with a new option for practical teaching, and PET imaging may provide additional options for pharmacology teaching activities in the future.

AUTHOR CONTRIBUTION

All authors approving the final content of the manuscript. L.G. wrote the manuscript. L.G., G.S., Y.Z., and J.P. performed the research. G.S. and Y.Z. analyzed the data. L.W. and K.C. designed the study.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

DATA AVAILABILITY STATEMENT

The data sets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.