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The effect of P-glycoprotein on the brain uptake of [^{18}F]Mefway in rodents

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The effect of P-glycoprotein on the brain uptake of [^{18}F]Mefway in rodents

Directed by Professor Young Hoon Ryu

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Jin Chung

December 2016

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ABSTRACT

The effect of P-glycoprotein on the brain uptake of [^{18}F]Mefway in rodents

Jin Chung

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Young Hoon Ryu)

I. INTRODUCTION

Efflux transporters in brain capillary endothelial cells impede xenobiotics, including therapeutic drugs and PET radioligands. Of these, P-gp (P-glycoprotein) and Bcrp (Breast cancer resistance protein) are prominent efflux pumps. P-gp is colocalized with Bcrp on the luminal membranes of brain capillary endothelial cells, and they have partly overlapping substrate specificities that include a wide range of compounds. Thus, to investigate the effects of P-gp, it is required to evaluate the impact of Bcrp. The aim of this study was to determine whether the brain uptake of [^{18}F]Mefway is influenced by the action of P-gp and Bcrp in rodents.

II. MATERIALS AND METHODS

To effectively suppress in vivo defluorination, fluconazole (OneFlu, JW Pharmaceutial, Seoul, Korea) was used. [^{18}F]Mefway was applied to the pharmacological inhibition rats and genetically disrupted mice. In rats, blocking of P-gp activity was performed by tariquidar (TQD). For the pharmacological inhibition experiments male Sprague-Dawley (n = 3 for each group) rats were

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used. For genetic disruption, male Mdr1a/b$^{(-/-)}$, Bcrp1$^{(-/-)}$, Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$, and wild-type (WT) mice were used (n = 4 for each group).

III. RESULTS

Pretreatment of TQD results in 160% higher hippocampal uptake compared with control rats. In genetically disrupted mice, a maximal brain uptake value of 3.2 SUV in the triple knockout mice (Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$) was comparable to that of the double knockout mice (Mdr1a/b$^{(-/-)}$) and two-fold those of the wild type and Bcrp1$^{(-/-)}$ knockout mice. The differences of binding values were statistically insignificant between control and experimental groups. The brain-to-plasma ratios for triple knockout mice were also 2-5 times higher than those for other groups.

IV. CONCLUSION

In PET, chemical knockout P-gp disruption induced considerable increase of brain uptake of [^{18}F]Mefway compared with control group or wild type. This result was substantiated by ex vivo experiments showing high brain-to-plasma ratios for P-gp disrupted mice. In conclusion, [^{18}F]Mefway is modulated by P-gp, and not by Bcrp in rodents.

Key words : [^{18}F]Mefway, P-glycoprotein, Breast cancer resistance protein, PET

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The effect of P-glycoprotein on the brain uptake of [^{18}F]Mefway in rodents

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(Directed by Professor Young Hoon Ryu)

I. INTRODUCTION

Multiple lines of evidence suggest that disturbance of serotonin 1A (5-HT$_{1A}$) receptors in the central nervous system is closely related to the etiology of various psychiatric disorders.[1] Assessment of integrity of 5-HT$_{1A}$ receptors is therefore an important subject in neuroscience.

Drug transporters belonging to the adenosine triphosphate binding cassette (ABC) family present in various tissues and play an important role in drug absorption and distribution.[2] Particularly, efflux transporters in brain capillary endothelial cells impede xenobiotics, including therapeutic drugs and PET radioligands.[3,4] Of these, P-glycoprotein (P-gp) and breast camcer resistance protein (Bcrp) are prominent efflux pumps. P-gp is colocalized with Bcrp on the luminal membranes of brain capillary endothelial cells,[5] and they have partly overlapping substrate specificities that include a wide range of compounds.[6,7] Thus, to investigate the effects of P-gp, it is required to evaluate the impact of Bcrp.

If one radiotracer is an avid substrate for these efflux transporters, the transporters work to limit the brain accessibility of that radiotracer and provide

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poor imaging signals. In this situation, reduced brain uptake results in either a small number of available receptors or active transporter function. Moreover, transporter activity has been described to change in neurologic diseases such as epilepsy and Alzheimer's disease.[8,9] This makes quantification of receptor binding more difficult if the radiotracer is a transporter substrate. Therefore, the evaluation of the effect of efflux transporters on the brain uptake of radiotracers is considered to be an important subject.

Positron emission tomography (PET) can non-invasively detect the receptor density in the plasma membrane due to its high sensitivity of 10^{-9} ~ 10^{-12} M.[10] The majority of radioligands for quantification of 5-HT_{1A} receptors have been developed from *N*-{2-[4-(2-methoxyphenyl)piperazinyl]ethyl}-*N*-2-Pyridylcyclohexanecarboxamide (WAY-100635). Although [*carbonyl*-^{11}C] WAY-100635, *trans*-4-^{18}F fluoro-*N*-2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide([^{18}F]FCWAY) and 4-^{18}F-fluoranyl-*N*-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-*N*-pyridin-2-ylbenzamide ([^{18}F]MPPF) have been applied to human subjects, these radiotracers had some limitations such as fast metabolism, metabolic instability and low penetration to the brain.[11-13] Later, [^{18}F]Mefway, which has comparably high selectivity and affinity to 5-HT_{1A} receptors and in vivo metabolic stability against radiodefluorination in humans, was developed to overcome these difficulties. Its efficacy in subjects ranging from rodents to humans has been previously demonstrated.[14-23]

In terms of permeability to drug transporters in the blood brain barrier (BBB), small chemical structure changes can induce a profound impact the susceptibility to brain efflux.[24] While [*carbonyl*-^{11}C]WAY-100635 was a weak

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P-gp substrate, benzyl substituted ([^{18}F]-MPPF) and reversed amide group analogue ([^{11}C]-(R)-RWAY) were identified as strong P-gp substrates.[25-27] Although [^{18}F]MPPF is identified as a strong P-gp substrate for rodent BBB, this seems not to be the case for primate BBB.[28] [^{18}F]Mefway is a WAY-100635 derivative and has an enlarged alkyl group compared to [^{18}F]FCWAY. Thus we assumed that [^{18}F]Mefway PET images could also be influenced by P-gp function. The purpose of the present study is to investigate the possible influence on P-gp on the brain uptake of [^{18}F]Mefway by pharmacological and genetically modulation of P-gp.

II. MATERIALS AND METHODS

1. General

All chemicals and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA). [^{18}F]fluoride ion was produced in the medical cyclotron (Eclipse HP, Siemens Ltd., Knoxville, TN, USA). Radioactivity was counted in a Capintec CRC-15R dose calibrator. Thin-layer chromatography was performed in Bioscan (Washington DC, USA). The crude product was purified by a high performance chromatograph (YL9100, Young Lin Instrument Co., Ltd, Anyang, Korea) with a reverse column (Xterra Prep, RP 18, 10 mm x 250 mm, Waters, Milford, MA, USA). To effectively suppress in vivo defluorination, fluconazole (OneFlu, JW Pharmaceutial, Seoul, Korea) was used. Tariquidar (TQD, MedKoo Bioscience , Chapel Hill, NC, USA) for injection was dissolved in propylene glycol, DMSO, and 5% dextrose solution (4:5:1) and immediately administrated to the rat. PET acquisition was carried out in an Inveon PET scanner (Siemens, Knoxville, TN, USA) This scanner has a peak absolute

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system sensitivity of ≥10 % in the 250–750 keV energy window, an axial field of view of 12 cm, 1.4-mm full width at half maximum (FWHM) spatial resolution at the center of the field of view, and a transaxial field of view of 10 cm. The PET images were analyzed with commercial software (PMOD, 3.1, PMOD Technologies Ltd., Adliswil, Switzerland). The chromatographic separation was performed with an Agilent (Palo Alto, CA) 1200 series HPLC system equipped with a Gemini C18 analytical column (50 × 2.0 mm, 3 μm i.d.; Phenomenex, Torrance, CA, USA) at a flow rate of 0.3 mL/min for 1.8 min. The MS/MS analysis was conducted using a tandem quadrupole mass spectrometer (Agilent 6460A, Palo Alto, CA).

2. Animals

For the pharmacological inhibition experiments male Sprague-Dawley (Raon Bio. Inc. Yong-in, Korea, body weight: 260 - 300 g, n = 3 for each group) rats were used. For genetic disruption, male Mdr1a/b$^{(-/-)}$ (FVB.129P2-Abcb1a^{tm1Bor}Abcb1b^{tm1Bor}, Bcrp1$^{(-/-)}$ (FVB.129P2-Abcg2^{tm1Ahs}), Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$ (FVB.129P2-Abcb1a^{tm1Bor}Abcb1b^{tm1Bor}Abcg2^{tm1Ahs}), and wild-type (WT) mice with FVB genetic background were obtained from Taconic Inc. (Germantown, NY, USA, body weight: 24 -30 g, n = 4 for each group). Mdr1a/b$^{(-/-)}$ knockout was created through sequential targeting of *Abcb1a* and *Abcb1b* in E14 embryonic stem cells (ES) cells. Resultant chimeras were backcrossed to FVB/N for seven generations (N7). Bcrp1$^{(-/-)}$ was created by targeting *Abcg2* in E14 ES cells derived from 129P2/OlaHsd mice and injecting the targeted cells into FVB blastocysts. Resultant chimeras were backcrossed to FVB/N for N7. Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$ was created through the

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crossbreeding of the Mdr1a/b targeted-mutation mouse and the Bcrp targeted-mutation mouse.

Animals were housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle and had free access to food and water. All animal studies were approved by the Committee for the Care and Use of Laboratory Animals at Yonsei University College of Medicine. All applicable institutional guidelines for the care and use of animals were followed.

3. Radiochemistry

[^{18}F]Mefway was synthesized by nucleophilic substitution reaction of *p*-tosylated precursor as previously described.[29] Specific activity at the end of synthesis was higher than 90 GBq/μmol as assessed by high performance liquid chromatography and radiochemical purity exceeded over 99 %.

4. Acquisition of PET Images

Animals were anesthetized with 2.5% isoflurane and catheters were inserted into the tail veins. Fluconazole (60 mg/kg) was infused at a constant rate to the veins of rats and mice for 1 h to suppress *in vivo* defluorination.[29] The body temperature was maintained at 36°C using the heating mat. The head of animal was placed in center of PET gantry. For rats, TQD (6 mg/kg) was bolusly injected to inhibit P-gp activity, followed by administration of radioactivity at a rate 1mL/min (12.4 ± 2.8 MBq). The PET response was the highest at this TQD dose, which is in good correspondence with previous findings.[30] For mice, radioactivity was injected solely to the tail at a rate 5ml/min (12.4 ± 2.8 MBq). PET images were acquired in the list mode for 90

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min (mice) or 120 (rats), then attenuation corrections were performed for only rat using data from a 10-min transmission scan with a ^{57}Co point source.

5. Image Analysis

Raw PET data were reconstructed in user-defined time frames (10 sec × 6 frames, 30 sec × 8 frames, 300 sec × 5 frames, 1800 sec × 2 frames for mice, 10 sec × 6 frames, 30 sec × 8 frames, 300 sec × 5 frames, 1800 sec × 3 frames for rats) with voxel dimensions of 0.86 × 0.86 × 0.80 mm^3 by a 2-dimensional order-subset expectation maximization (OSEM) algorithm. The acquired images were evaluated by region-of-interests (ROIs) analysis using PMOD 3.1. Regions of interests are the hippocampus and cerebellum. The cerebellum was used as reference tissue for kinetic modeling because this region is known to have very low concentration of 5-HT$_{1A}$ receptors in rodents. Time-activity curves (TACs) in these regions were acquired and regional TACs were normalized in units of standardized uptake value (SUV).

Individual binding value estimations were computed from multilinear reference tissue model (MRTM).[31] Then with these estimated k_2's, regional distribution volume ratios (DVR) were calculated by using the non-invasive Logan's graphical analysis method.[32] Estimated non-displaceable binding potentials (BP_{ND}) were calculated as DVR - 1.

6. Ex Vivo Quantification of Mefway

After completion of PET, the mice were awakened from anesthesia and returned to their cage until the ex vivo quantification of Mefway. Animals were intravenously administered cold Mefway at 5 mg/kg. After 1h, the mice were

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euthanized, and the blood and brain were collected immediately. After washing, the tissues were added to five volumes of phosphate buffered saline per weight, homogenized, and stored at –20°C until analyzed for Mefway by LC-MS/MS.

The concentration of cold Mefway was determined by a LC-MS/MS method developed in our laboratory. Briefly, 30-μL plasma samples were mixed with 270-μL of acetonitrile–methanol mixed solution (1:1, v/v) containing 20 ng/mL of the internal standard (IS), gliclazide and centrifuged (15,800g, 10 min). The clear supernatants were transferred to LC-MS/MS vials, and then a 5-μL aliquot was injected into the LC-MS/MS system. Multiple reaction monitoring modes were used for the quantification: *m*/*z* 455.3 → 263.1 for Mefway and *m*/*z* 324.1 → 110.1 for gliclazide (internal standard). The fragmentor energy of the MS was set at 155 and 115V for Mefway and gliclazide, respectively. The peak areas of all samples were integrated automatically using MassHunter program (Agilent Inc.).

7. Statistical Analysis

All data are presented as mean ± standard error of the mean (SEM). Differences in BP_{ND} values were analyzed with the student t-test. All statistical analyses were performed with Prism 5 (ver. 5.04, GraphPad, La Jolla, CA, USA).

III. RESULTS

1. Pharmacological inhibition of P-gp

Representative PET summation images in the rat brain showed that pharmacological inhibition of P-gp using TQD resulted in apparent increment of

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brain radioactivity (Fig. 1).

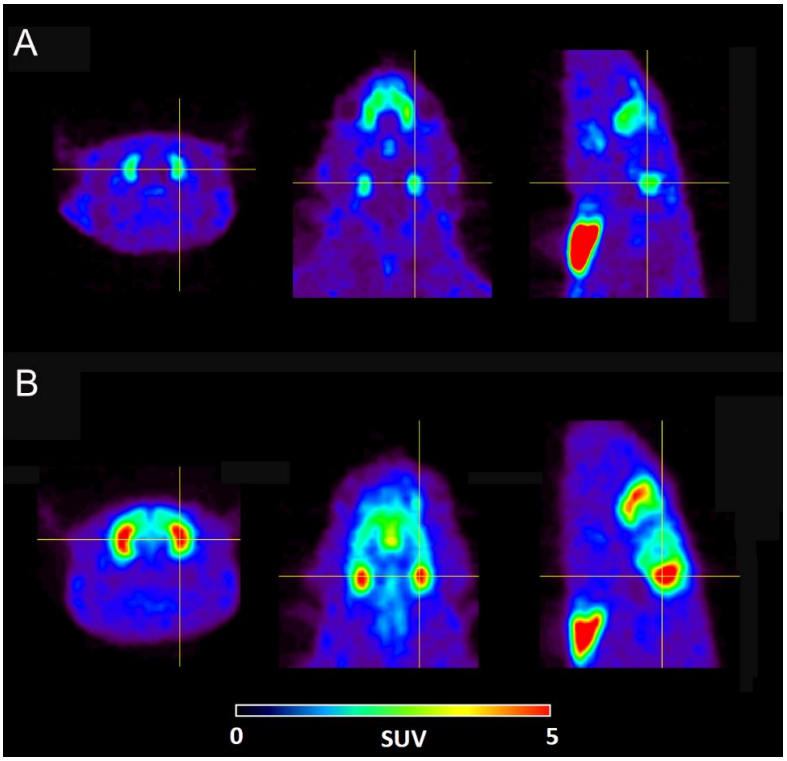

Figure 1. Representative PET images in the rat brain. Control group (A) and phamacologogical inhibition group (B). Shown are time-averaged images from the brain between 0 - 90 min. *Cross* indicates the hippocampus.

In the control rats, hippocampal and the cerebellar uptakes peaked early (> 5 min) and were 3.6 SUV and 2.1 SUV in control rats, respectively. In the P-gp blocked rats, radioactivities in the hippocampus and the cerebellum were 2.2- and 3.9-fold those of the control group, respectively
(Fig. 2).

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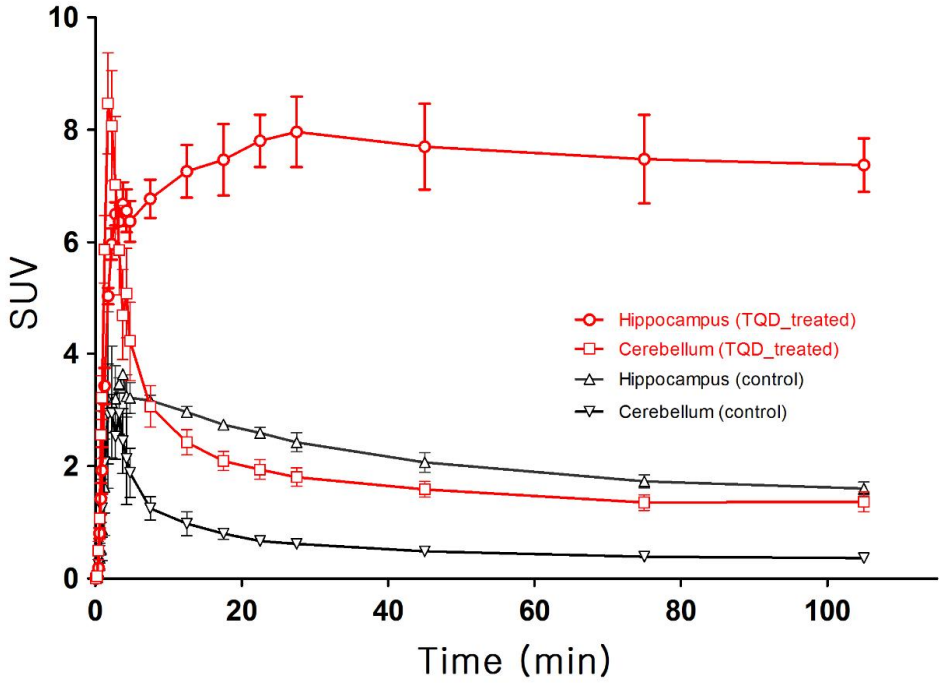

Figure 2. Regional time-activity curves before and after administration of tariquidar (TQD). Red line means TQD pretreated group and black line shows the control group. Data represent mean ± standard error of the mean (SEM).

Quantitative analysis using the non-invasive Logan's graphical method resulted in comparable mean values for the BP_{ND} (control group: 3.13 vs. TQD pretreatment = 3.46, respectively, Table 1).

Table 1. BP_{ND} values in the rats and the mice

Rat		Mice			
Control	P-gp inhibition	Wild type	Bcrp(-/-)	Mdr1a/b(-/-)	Mdr1a/b(-/-) Bcrp(-/-)
3.13 ± 0.29	3.46 ± 0.35	1.64 ± 0.14	1.65 ± 0.17	1.83 ± 0.26	1.73 ± 0.31

Data are presented as mean ± SD

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The difference of binding values was not statistically significant. The kinetic analysis resulted in comparable mean values for the BP_{ND} in the P-gp and/or Bcrp disrupted mice: (BP_{ND} values: Mdr1a/b$^{(-/-)}$ = 1.83 , Mdr1a/b $^{(-/-)}$/Bcrp1$^{(-/-)}$ = 1.73, Bcrp1$^{(-/-)}$ = 1.65, and WT = 1.64, respectively, Table 1).

2. Genetically disrupted mice

As shown in Fig. 3A, genetically P-gp disrupted mice (i.e., Mdr1a/b$^{(-/-)}$ and Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$ showed considerably higher brain uptakes compared with WT or Bcrp1$^{(-/-)}$. The highest hippocampal uptake was observed in Mdr1a/b$^{(-/-)}$Bcrp$^{(-/-)}$ mice and these uptake values were not statistically different from Mdr1a/b$^{(-/-)}$. Peak uptake values for Mdr1a/b$^{(-/-)}$ and Mdr1a/b$^{(-/-)}$Bcrp$^{(-/-)}$ were about 1.7-fold those in the WT and Bcrp1$^{(-/-)}$ mice ($p < 0.001$) whereas, the uptake differences between Bcrp1$^{(-/-)}$ and WT were insignificant ($p = 0.62$, Fig. 3B).

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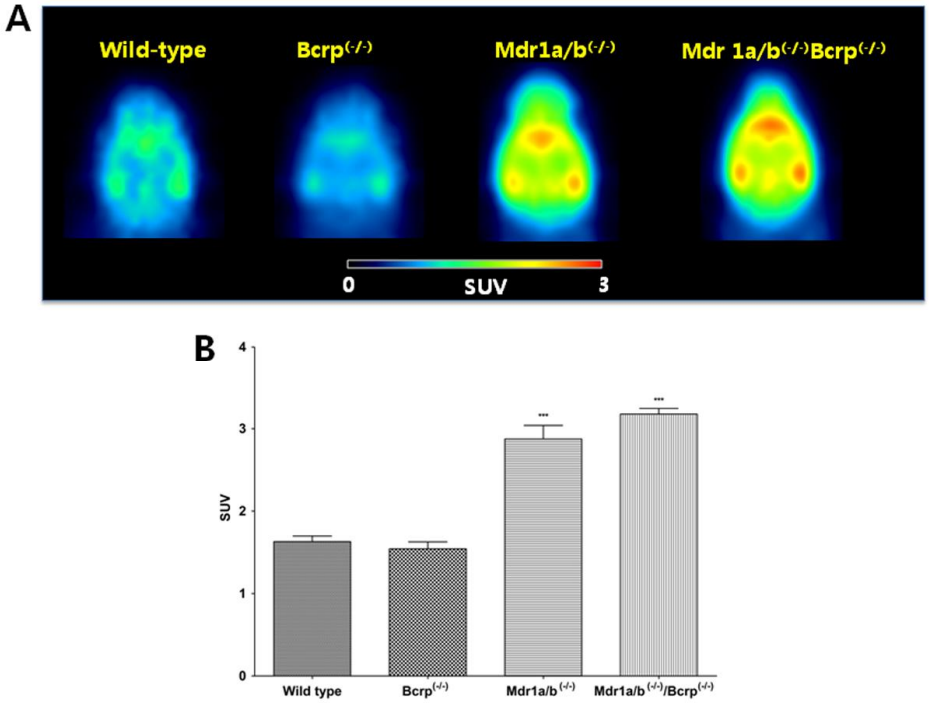

Figure 3. Coronal PET summation and peak uptake values at 10 min in the transgenic mice. (A) Coronal PET summation images (0 - 60 min) of wild-type, Bcrp1$^{(-/-)}$, Mdr1a/b$^{(-/-)}$, and Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$. (B) Peak uptake values at 10 min in the transgenic mice (n= 3). Mean bars indicate the significant differences of the hippocampal uptake between Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$ or Mdr1a/b$^{(-/-)}$ and wild-type ($^{***}P$ <0.001). However, there was no significant difference between Bcrp1$^{(-/-)}$ and wild-type (p = 0.62). Data represent mean ± standard error of the mean (SEM).

The kinetic analysis resulted in comparable mean values for the BP_{ND} in the P-gp and/or Bcrp disrupted mice: (BP_{ND} values: Mdr1a/b$^{(-/-)}$ = 1.83, Mdr1a/b$^{(-/-)}$/Bcrp1$^{(-/-)}$ = 1.73, Bcrp1$^{(-/-)}$ = 1.65, and WT = 1.64, respectively, Table 1).

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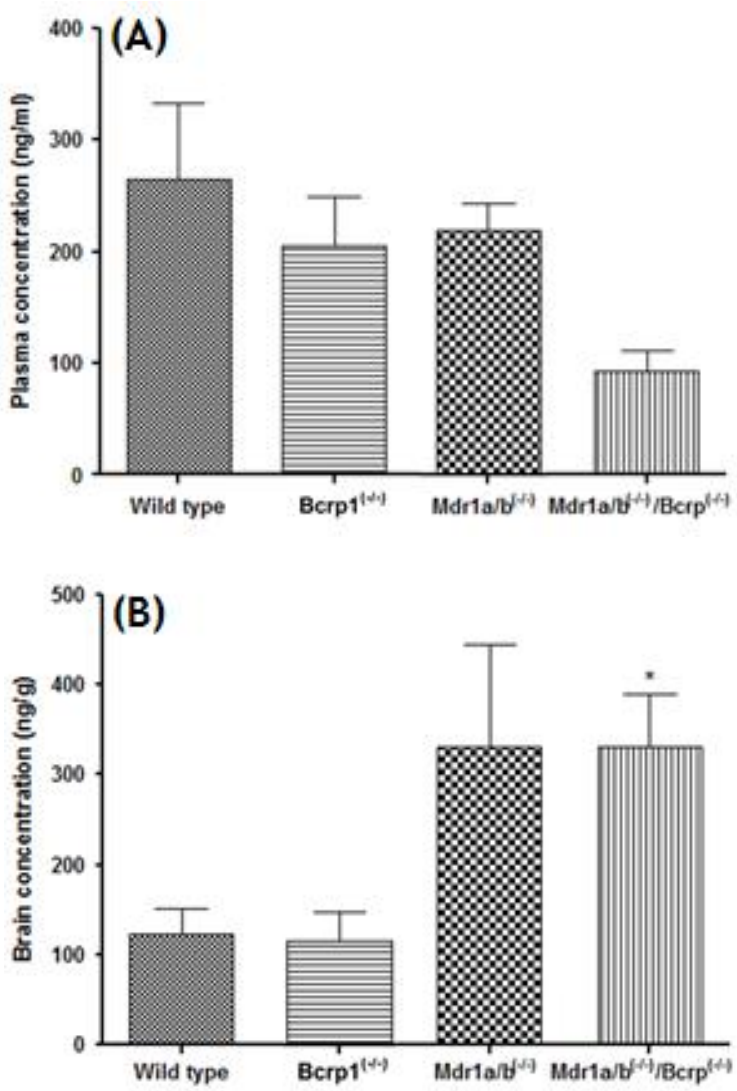
3. Brain-to-plasma ratio of Mefway

The brain-to-plasma ratios of P-gp disrupted groups were significantly higher than those in the WT and Bcrp1$^{(-/-)}$ groups ($p = 0.048$ for Mdr1a/b$^{(-/-)}$ and $p < 0.001$ Mdr1a/b$^{(-/-)}$Bcrp$^{(-/-)}$ compared with WT, Fig. 4).



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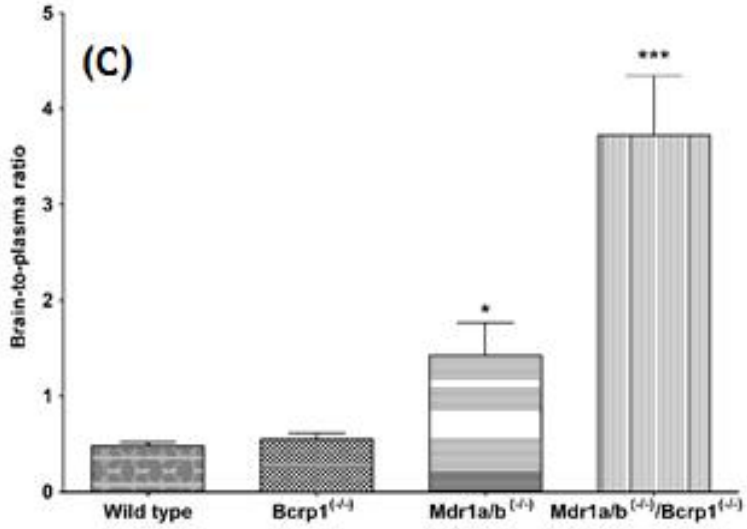

Figure 4. Comparison of Mefway concentration in plasma (A), brain (B), and brain-to-plasma ratio (C). Mean bars indicate the significant differences in the Mdr1a/b$^{(-/-)}$ ($^{*}p = 0.048$) or in the Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$ ($^{***}P < 0.001$) compared with wild-type. Data represent mean ± standard error of the mean (SEM).

The substantially higher ratio in Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$ mice compared to Mdr1a/b$^{(-/-)}$ was likely caused by the widespread distribution of P-gp in tissues other than the brain including the intestine, liver, and kidneys .

IV. DISCUSSION

In the present study, we evaluated the effect of efflux transporters on the brain uptake of [^{18}F]Mefway in the rodent brain. After pharmacological modulation of P-gp activity, radioactivities in the brain was significantly elevated compared with the control group. In the genetic inhibition model, P-gp knockout groups also showed higher brain uptake value than Bcrp knockouts or wild type. These results are in accordance with ex vivo experiments showing

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the high brain concentration in P-gp knockout mice. Taken together, our results suggest, for the first time to our knowledge, that [^{18}F]Mefway is a P-gp substrate, not Bcrp in the murine BBB.

To investigate the effects of P-gp on the brain uptake of [^{18}F]Mefway, we performed PET studies using different rodent models: chemical inhibition and genetic knockout systems. For chemically knocked out rats, we used tariquidar as a P-gp specific modulator because this intravenous administration of TQD in rats induces fast and long inhibition of P-gp in the absence of interaction with cytochrome P450 activity.[33] The first- and second- generation P-gp inhibitors such as verapamil, cyclosporine A, valspodar have low selectivity and binding affinity to P-gp.[34] However, the pharmacological blockade of P-gp using TQD induces inhibits Bcrp activity.[35] Hence, we used genetic inhibition mice. In the present study, the highest brain uptake value in P-gp/Bcrp knockout mice can be accounted for by synergistic effects. PET results were quite in opposition to an in vitro bidirectional transport assay showing that ^{18}F-Mefway was not a substrate for P-gp (data not shown). This discrepancy could be explained in that an in vitro bidirectional transport assay is not appropriate for drugs that quickly permeate cell monolayers.[36]

To evaluate the differences of binding potential, we used non-invasive Logan's graphical analysis. Compartment modeling with the arterial input function is considered as the gold standard in neuroreceptor PET analysis generating the ratio of tracer concentration between plasma and the brain in the equilibrium state. However, this method is highly influence by the action of P-gp. Before a substrate completely crosses the BBB, a P-gp efflux pump transports some extent of its substrate from the endothelial membrane to the

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plasma. This P-gp action causes either increased efflux from or decreased transport to the brain.[26] Moreover, the plasma concentration of the substrate is often affected by this efflux pump.[37] On the other hand, the reference model is much less sensitive to P-gp activity. In the present study, the differences of BP_{ND} values between the control and experiment groups were comparable. These results imply that P-gp function is similar in both the receptor rich and poor region. This P-gp activity agrees that comparable binding values in the striatum and the cerebellum for [^{18}F]Fallypride in rodents.[38]

The fact that [^{18}F]Mefway is a P-gp substrate in rodents is likely indicative of similar characteristics in higher species, but further investigation is required. Species differences in TQD induced P-gp modulation exists. In fact, several radioligands such as [^{11}C]verapamil, [^{11}C]flumazenil, [^{11}C](R)-RWAY, and [^{18}F]altanserin known as P-gp substrates in rats have displayed sufficient brain uptake in humans.[39] Uchida et al. suggested that different degrees of uptake in primates compared to rodents are strongly influenced by differences in transporter expression at the BBB.[40] Based on these perspectives, [^{18}F]Mefway may not be affected by P-gp in higher species. From only genetic disruption PET results, we cannot absolutely exclude effect of Bcrp on the brain uptake of [^{18}F]Mefway. Further studies are needed to assess this issue.

Although this study is lack of radiochromatographic and plasma protein binding analysis, we think that these issues are not critical for evaluation of P-gp on the brain uptake for [^{18}F]Mefway. The previous work demonstrated that [^{18}F]-trans-4-fluoromethylcyclohexane carboxylate as a major metabolite of [^{18}F]Mefway was extensively localized in peripheral regions.[20] In addition, if majority of a TQD is bound to rodent plasma proteins and plasma free fraction

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of [^{18}F]Mefway is large, the enhancement of brain uptake can be attributed to the effect of TQD. However, this hypothesis are unlikely because a plasma free fraction of [^{18}F]Mefway was quantified of 5 % or 15 % in monkeys and 5 % in humans, respectively.[17,41] Genetically disrupted mice also showed the increment of brain uptakes in the P-gp knockout mice.

Even if [^{18}F]Mefway is a substrate for P-gp in the rodent brain, this radioligand may be a candidate for clinical studies. Firstly, [^{18}F]Mefway showed less susceptibility to Bcrp activity. In humans, Bcrp was expressed at the BBB significantly more highly than P-gp based on mRNA and proteomic analysis.[40,42] Moreover, [^{18}F]Mefway has a higher target-to-reference value than [^{18}F]MPPF and considerably greater metabolic stability against radiodefluorination than [^{18}F]FCWAY in humans.[14,17,43,44]

V. CONCLUSION

In [^{18}F]Mefway PET, chemical knockout P-gp disruption induced considerable increment of brain uptakes compared with control group or wild type. This result was substantiated by ex vivo experiments showing high brain-to-plasma ratios for P-gp disrupted mice. These studies demonstrated that [^{18}F]Mefway is a substrate for P-gp in rodent brain.

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ABSTRACT(IN KOREAN)

설치류의 [^{18}F]Mefway에서 뇌 섭취에 기여하는 P-glycoprotein의 효과

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정진

I. 목적

뇌 모세혈관 내피 세포에서 유출 운반자들은 치료 약물들과 PET 방사성 리간드들을 포함한 생체이물질들을 방해한다. 이들 중에서 P-glycoprotein (P-gp)와 breast cancer resistance protein (Bcrp)는 가장 중요한 유출 운반자들이다. P-gp는 뇌 모세혈관 내피 세포의 관강내막에 Bcrp와 함께 발현이 되어 있으며 일부 기질 특이성을 공유하고 있다. 따라서 P-gp의 효과를 알아보기 위해서는 Bcrp의 효과를 알아볼 필요성이 있다. 본 연구의 목적은 설치류의 [^{18}F]Mefway에서 P-glycoprotein (P-gp)와 breast cancer resistance protein (Bcrp)의 뇌섭취에 관련된 효과를 알아보고자 하였다.

II. 대상 및 방법

탈불소화를 효과적으로 억제시키기 위하여 fluconazole (OneFlu, JW Pharmaceutial, 서울, 대한민국)를 사용하였다. [^{18}F]Mefway를 약물학적으로 억제시킨 쥐와 유전적으로 변형시킨 생쥐에게 적용을 하였다. 쥐에서는 tariquidar(TQD)를 이용하여 P-gp 기능을 억제시켰다. 약물학적으로 억제시키는 실험을 위해서는 수컷 Sprague-Dawley (각 그룹 당 세 마리) 쥐를 사용하였다. 유전적 변형을

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위하여서는 수컷 Mdr1a/b$^{(-/-)}$, Bcrp1$^{(-/-)}$, Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$, 그리고 대조군 생쥐가 사용되었다 (각 그룹 당 네 마리).

III. 결과

TQD를 전처치함으로써 대조군 쥐와 비교 시 해마의 뇌 섭취가 160% 증가되었다. 유전적으로 변형된 생쥐에서, 최대 뇌 섭취가 3.2 SUV인 삼중 유전자 결손 생쥐 (tKO, Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$)와 비교 시 이중 유전자 결손 생쥐 (dKO, Mdr1a/b$^{(-/-)}$) 와 최대 뇌 섭취가 비슷하였으며 대조 군과 Bcrp1$^{(-/-)}$ 유전자 결손 생쥐와 비교 시 두 배 높은 최대 뇌 섭취를 보였다. 대조 군과 실험 군간의 결합 가치의 차이는 통계적으로 의미가 없는 것으로 나타났다. [^{18}F]Mefway 농도의 뇌와 혈장간 비율은 다른 그룹과 비교 시 삼중 유전자 결손 생쥐에서 2.5배 높은 것으로 확인되었다.

IV. 결론

PET에서 화학적으로 P-gp 결여된 경우 대조군과 비교시 [^{18}F]Mefway의 뇌에서 섭취도가 매우 많이 증가되었다. 이러한 결과는 P-gp 결손 생쥐에서도 높은 뇌-혈장간 비율을 보인 실험 결과에서도 입증되었다. 결론적으로 [^{18}F]Mefway는 설치류에서 Bcrp가 아닌 P-gp에 의해서 조절이 된다.

핵심되는 말 : [^{18}F]Mefway, P-glycoprotein, 유방암 저항성 단백질 (Breast cancer resistance protein), 양전자 단층촬영 (PET)