Uptake of Aβ by OATPs Might Be a New Pathophysiological Mechanism of Alzheimer Disease

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Research Article

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Abstract

Background

The accumulation of neurotoxic amyloid-beta (Aβ) in the brain is a characteristic of Alzheimer's disease (AD). Over the last decade, a number of reports have shown that P-glycoprotein (encoded by *ABCB1*) actively mediates the efflux transport of Aβ peptides. However, the mechanism by which Aβ peptides enter the cells is not clear. We found that the protein expression oforganic anion transporting Polypeptide 2 (Oatp2) in the liver tissue of mice with AD was significantly higher than that in the normal mice. In contrast, the protein expression of Oatp2 in the brain significantly decreased in mice with AD. OATP1B1, an important drug transporter might be related to the pathophysiology of AD.

Results

In this study, we established an OATP1B1-GFP-HEK293T cell model to confirm the OATP1B1 mediated transport of$A\beta_{1-42}$.Compared to the control group of GFP-HEK293Tcells, the uptake of $A\beta_{1-42}$ protein in the OATP1B1-GFP-HEK293T group increased significantly with the increase in concentration of $A\beta_{1-42}$, and also increased significantly with an increase in the duration of incubation. Similar results were observed in the flow cytometry experiment, and the uptake of $A\beta_{1-42}$in HEK239T-OATP1B1 cells was almost twice that in the control group. These results indicate that OATP1B1 acts as an important “carrier” for the transport of $A\beta_{1-42}$ from the blood to the tissues, including liver and brain.

Conclusions

This is a novel and interesting finding and OATP1B1 can be investigated as a new treatment target for AD.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive loss of memory and acquired knowledge, until the complete loss of activities of daily life. Alzheimer's disease endangers the health of the elderly following cardiovascular disease, cerebrovascular disease, and tumor [1]. According to the data released by the International Alzheimer's Association (ADI) in 2018, there are currently about 44.40×10^6 patients with AD in the world, with an average of 1 new case detected every 3 seconds [2]. According to the statistics of the World Health Organization, AD will become the fourth highest cause of disease burden in China by 2020. This could lead to a heavy healthcare burden on the society and families, and develop into a serious social and health problem.

Although many scholars think that neurotoxicity caused by amyloid-beta (Aβ) is one of the main causes of AD, the exact pathogenesis of AD remains elusive. Currently, there is no effective method to treat or prevent the progression of AD. Therefore, it is imperative to understand the etiology, pathogenesis, and treatment target for AD as soon as possible, and to discover safe and effective treatment approaches in the field of AD research. It has been suggested that the imbalance between the clearance of Aβ and its accumulation in the peripheral and central systems is at the core of the development of AD [3]. However, the mechanism for correcting this imbalance is not clear. In recent years, drug transporters have played a very important role in the development of drugs, endogenous substances, and disease pathophysiology. Are these transporters related to the occurrence and development of AD? It has been shown that P-glycoprotein (P-gp□ABCB1) is an important barrier for the peripheral and central clearance of Aβ [4,5], and these exosomes can reduce the accumulation of Aβ in the tissues. However, a previous study showed that the ATPase activity measured in the vesicles of the plasma membrane of K562 cells overexpressing P-gp was not increased by the presence of $A\beta_{42}$, suggesting that $A\beta_{42}$ is not a P-gp substrate [6]. Similarly, P-gp of pirarubicin was unaffected by the expression of $A\beta_{42}$. Moreover, the overexpression of P-gp does not protect the cells against $A\beta_{42}$ toxicity[6]. Considered together, these results indicate that $A\beta_{42}$ is not transported by P-gp [6].Therefore, although much evidence from human, animal, and in vitro studies has examined the contribution of P-gp in the clearance of Aβ, the role of P-gp in AD is still contentious [7].

All previous studies focused on the efflux transporter of P-gp, but whether uptake transporters play a role in the pathophysiology of AD is unknown. As an important member of the solute delivery protein family, OATP has a wide range of substrates, including a variety of internal and external substances, especially the process of drugs in vivo, and its coding genes are collectively referred to as *SLCO* genes [8]. Among them, the specific expression of OATP1B1 (mouse hepatocyte expression homologous gene Oatp2) in the basement membrane of human hepatocytes has been widely studied, and its mediated substrate transport (drug) is very extensive [9]. Our

research group tried to explore whether there is a relationship between OATPlevels and AD. In a previous study, we analyzed the brain and liver tissue of mice with AD, and found that the protein expression of Oatp2 in the brain significantly decreased in the affected mice [9]. In contrast, the protein expression of Oatp2 in the liver tissue of mice with AD was significantly higher than that in the normal mice [9]. Results for the mRNA expression of Oatp2 showed that compared to that in the normal mice, it was significantly lower in the brain but significantly higher in the liver tissue in mice with AD [9]. However, the study could not confirm the relationship between Oatp2 and AD. Therefore, this study aimed to confirm whether OATPs mediate the transport of $A\beta_{1-42}$. This is a very novel and interesting study that might open a new door for research on the pathophysiology of AD.

2. Materials And Methods

2.1 Materials and main instruments

0.45 μm PVDF membrane,Millipore Inc. (Massachusetts, USA); Skimmed milk powder, Yili Industrial Group Co., Ltd. (Hohhot, China). BEYOCOLOR color pre-dyed protein molecular weight standard: Fermentas Inc(Canada); ECL plus luminescent kit, SDS-PAGE protein sample buffer (5 ×), Western and IP cell lysate, PMSF and BCA protein concentration determination kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China); Tris HCl / SDS (1.5 mm, pH 8.8) and Tris HCl / SDS (0.5 mm, pH 6.8) were purchased from Shanghai Biotechnology Co., Ltd.; 30% acrylamide/bis solution, glycine were purchased from Bio-RAD (California, USA); Tris alkali, SDS and ammonium persulfate were purchased from BiosharpInc.;$A\beta_{1-42}$, BiosharpInc. (bs0076R, Hefei, China)Goat anti mouse IgG,Allied biology company(GAM007, Shenzheng, China); Goat anti rabbit IgG, Allied biology company (GAR007, Shenzheng, China); β-actin,Allied biology company (ab008, Beijing, China); HEK293T cells and OATP1B1 virus, Hangzhou HibioTechnology Co.,Ltd. (Hangzhou, China);fetal bovine serum, GibcoInc. (California, USA); Rapid total RNA Extraction Kit, Shanghai Jierui Bioengineering Co., Ltd. (Shanghai, China); The reverse transcription kit (HiScript II Q RT SuperMix for qPCR) and Quantitative PCR kit (ChamQTM SYBR Color Qpcr Master Mix)Vazyme Biotech Co.,Ltd. (Nanjing, China).

Flow cytometer: Becton,Dickinson and Company (New Jersey, USA); Cell incubator, Thermo Fisher Scientific (Massachusetts, USA); Inverted microscope, Olympus company (Japan). Desktop low-speed centrifuge, Shanghai medical equipment (Group) Co., Ltd. (Shanghai, China); Mini-Proten Tetra System, Bio-RAD, (California, USA); ChemiDoc XRS+ System, Bio-RAD (California, USA); Low light spectrophotometer, Beijing Meilin Hengtong Technology Co., Ltd. (Beijing, China).

2.2 Methods

HEK293T cells and OATP1B1 virus were obtained from Hangzhou HibioTechnology Co.,Ltd. (Hangzhou, China). The OATP1B1-GFP-HEK293T and GFP-HEK293T cell models were established as previously described [8]. The OATP1B1 sequence was synthesized into a pEGFP-N1 vehicle.Then, the vehicle was transfected into DH5α competent cells to generate more pEGFP-N1-OATP1B1 plasmids. Finally, the pEGFP-N1-OATP1B1 plasmid was transfected into HEK293 cells. Then, qPCR and western blot testing were used to detect the expression of OATP1B1 in the cells [8]. The complete RNA was extracted using a Trizol centrifugal column, and reverse transcription was carried out to obtain the cDNA. Finally, a PCR system was established, and the mRNA levels ofOATP1B1-GFP-HEK293T cells and GFP-HEK293T cells were analyzed using a real-time quantitative PCR detection system. Primers for OATP1B1 were OATP1B1-F: AACTCCTACTGATTCTCGATGGG; OATP1B1-R: GTTTCCAGCACATGCAAAGAC; actin-F: TGACGTGGACATCCGCAAAG; actin-R:CTGGAAGGTGGACAGCGAGG. OATP1B1-GFP-HEK293T cells and the control cell line GFP-HEK293T were used to explore the uptake features of $A\beta_{1-42}$. All HEK293 stable cell lines were maintained in dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum, 1% antibiotic, and antimycotic solution, and 600 μg.ml-1 geneticin. The cell lines were cultured in a humidified atmosphere (95% O2, 5% CO2) at 37°C. $A\beta_{1-42}$was dissolved in dimethylsulfoxide (DMSO) and cell toxicity was performed to select reasonable concentrations for the uptake experiments. Next, a series of concentrations of $A\beta_{1-42}$ (0, 0.4, 1.0, and 2.5μM) were added to the OATP1B1-GFP-HEK293T and GFP-HEK293 cells and incubated for approximately 24, 48, and 72 h. Then, the cells were washed with ice-coldphosphate buffer saline (PBS) 3× and lysed with cell lysis buffer. Cell lysis buffer was collected and centrifuged at 1.4×104 rpm for 20 min. The supernatants were used to analyze the $A\beta_{1-42}$ by western blotting (WB). At the same time, the cells were collected to detect the uptake of $A\beta_{1-42}$ in both OATP1B1-GFP-HEK293T and GFP-HEK293 cells by flow cytometry.

2.3 Statistical analysis

All statistical analyses were performed using Student's t-test and one-way ANOVA, with SPASS 13.0. Data are presented as mean ±standard deviation from at least three separate experiments. * and ** indicate $p<0.05$ and $p<0.01$, respectively. P value less than 0.05

was considered statistically significant. Western blot bands were calculated using Image J software (Java 1.6.0_20, NIH, USA).

3. Results

We successfully created an OATP1B1-GFP-HEK293T cell model and compared it to the control. OATP1B1 increased by approximately 214% in the OATP1B1-GFP-HEK293T cells (Figure 1). The qPCR results also showed that the mRNA expression of OATP1B1 in the OATP1B1-GFP-HEK293T cells was significantly higher than that in the GFP-HEK239T cells (1.03±0.22 vs 0.00±0.00). After treatment with$A\beta_{1\text{-}42}$ (0, 0.4, 1, 2.5 μM) for 24, 48, and 72 h, by using western blotting, the uptake of $A\beta_{1\text{-}42}$ inOATP1B1-GFP-HEK293T cells significantly increased with increasing$A\beta_{1\text{-}42}$ concentration and the duration of incubation. Similar results were also seen in the HEK293T cells; however, OATP1B1-GFP-HEK293T cells mediated uptake of $A\beta_{1\text{-}42}$ was higher than that of GFP-HEK293 cells, especially when the incubation time was 72 h (Figure 2). From the gray value of the western blot, the results showed that $A\beta_{1\text{-}42}$ uptake in GFP-HEK239T cells and OATP1B1-GFP-HEK293T cells was 0.11 vs 0.10, 0.38 vs 0.52, 0.56 vs. 0.83, 0.62 vs 0.93 when the cells were treated with $A\beta_{1\text{-}42}$ (0, 0.4, 1, and 2.5 μM) for 24 h, while theywere 0.09 vs 0.12, 0.40 vs 0.87, 0.46 vs 0.97, 0.68 vs 1.24 for 48 hours, and 0.08 vs 0.07, 0.47 vs 0.66, 0.69 vs 1.49, 0.92 vs. 2.16 for 72 h (Figure 3).

From the results of flow cytometry (Figure 4), we observed thatthe uptake of $A\beta_{1\text{-}42}$ increased both GFP-HEK239T and OATP1B1-GFP-HEK293T cells, and compared to the HEK293T cells, the uptake of $A\beta_{1\text{-}42}$ in OATP1B1-GFP-HEK293T cells increased significantly with the increase in the duration of incubation (Figure 5 and Table 1). By calculating the fluorescence intensity of $A\beta_{1\text{-}42}$ in the GFP-HEK239T and OATP1B1-GFP-HEK293T cells, it is apparent that the intensity of $A\beta_{1\text{-}42}$ in OATP1B1-GFP-HEK293T cells was higher than that in GFP-HEK239T cells. The results are shown in Figure 6 and Table 2. Results of both western blotting and flow cytometry confirmed that $A\beta_{1\text{-}42}$ was the substrate of OATP1B1. OATP1B1 is involved in the transport of $A\beta_{1\text{-}42}$ in tissues.

4. Discussion

One study demonstrated that endocytosis is the major, if not the only pathway for the entry of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ into the SH-SY5Y cells [10]. This disputes some previous beliefs about passive diffusion or membrane-penetration modes of entry, which would allow Aβ direct access to the cytoplasm [11]. Simultaneously, the clearance of cerebral Aβ is a complex process mediated by various systems and cell types, including vascular transport across the blood–brain barrier, glymphatic drainage, and engulfment and degradation by the resident microglia and infiltrating innate immune cells[12]. Therefore, the process for the uptake and efflux of Aβ is complex and chaotic. Our study showed that $A\beta_{1\text{-}42}$ was the substrate of OATP1B1, which is a novel and interesting finding. As an uptake transporter that might play an important role in the cellular uptake of $A\beta_{1\text{-}42}$, OATP1B1will become a new target for the treatment of AD. We found that the protein expression of Oatp2 (OATP1B1) in the liver tissue of mice with AD was significantly higher than that in the normal mice. In contrast, the protein expression of Oatp2 in the brain was significantly lower in mice with AD [9]. OATP1B1 might have a “self-defense system role” that could decrease the uptake quantity of $A\beta_{1\text{-}42}$ in the blood and decrease the uptake of $A\beta_{1\text{-}42}$ in brain. At the same time, it is necessary to confirm whether other members of the OATPs families act as a “carrier” of $A\beta_{1\text{-}42}$. Despite decades of research, the pathophysiology of AD remains elusive. Understanding the normal versus impaired processing and clearance mechanisms affecting Aβ peptides will assist in the development of more effective therapeutic agents to combat this progressive neurodegenerative condition that continues to devastate millions of patients globally [7].We hope that this study will be helpful in the research for the pathophysiology of AD. The active uptake pathway for the entry of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ into the nerve cells by the OATPs is likely to become a new and novel mechanism of the pathophysiology of AD.

Abbreviations

AD: Alzheimer's disease;organic anion transporting Polypeptide: OATP; Aβ: amyloid-beta ;

Declarations

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. The study design was approved by the appropriate ethics review board. All authors have approved the manuscript for submission. We have read and understood your journal’s policies, and we believe that neither the manuscript nor the study violates any of these. The authors declare that they have no conflicts of interest.

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Authors' contributions

Jinhua Wen and Ying Zhou designed and conducted the experiment; Yanni Lv and Menghua Zhao completed the experiments of western blot and flow cytometry. Jinhua Wen wrote the manuscript.

Availability of data and materials

The data used can be found in Additional file.

Consent for publication

Not applicable.

Competing interests

We declare that they have no conflicts of interest.

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Tables

Table 1 The cellular uptake of $A\beta_{1-42}$ in GFP-HEK239T cells and OATP1B1-GFP-HEK293T cells following incubation with 0.4, 1, 2.5 μM $A\beta_{1-42}$ for 24, 48, 72h, respectively.

	$A\beta_{1-42}$ 0μM		$A\beta_{1-42}$ 0.4μM		$A\beta_{1-42}$ 1μM		$A\beta_{1-42}$ 2.5μM	
	NC	OATP1B1	NC	OATP1B1	NC	OATP1B1	NC	OATP1B1
24h	0.52±0.05	0.47±0.12	9.16±0.93	10.88±0.31*	16.28±2.42	24.50±4.48*	36.01±4.25	42.00±4.27
48h	0.62±0.11	0.67±0.15	12.18±0.25	19.87±1.70**	20.07±0.98	49.42±3.02**	37.04±1.77	65.38±4.52**
72h	0.52±0.07	0.63±0.10	16.92±0.20	29.53±2.58**	24.61±4.57	56.60±8.03**	44.39±5.76	84.13±3.03**

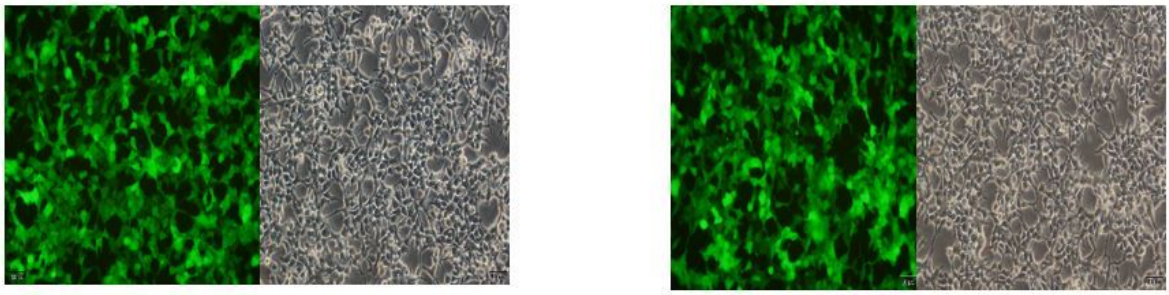
NC: GFP-HEK239T cells ; OATP1B1:OATP1B1-GFP-HEK293T cells.*P<0.05;**P<0.01.

Table 2 Fluorescence intensity of $A\beta_{1-42}$ in the GFP-HEK239T and OATP1B1-GFP-HEK293T cells when the cells were treated with different concentration of $A\beta_{1-42}$ during the different incubation time.

	Aβ1-42 0μM		Aβ1-42 0.4μM		Aβ1-42 1μM		Aβ1-42 2.5μM	
	NC	OATP1B1	NC	OATP1B1	NC	OATP1B1	NC	OATP1B1
24h	4162±336	3943±167	4386±63	4316±52	4316±107	95484±20582	25148±7713	60935±4789
48h	3980±145	4100±566	4333±19	4356±74	4440±323	58629±12127	51704±9795	139172±18360
72h	4115±225	4233±562	4335±52	38803±2597	16363±8540	126123±21186	68502±19324	166943±20123

NC:GFP-HEK239T cells ; OATP1B1:OATP1B1-GFP-HEK293T cells.

Figures



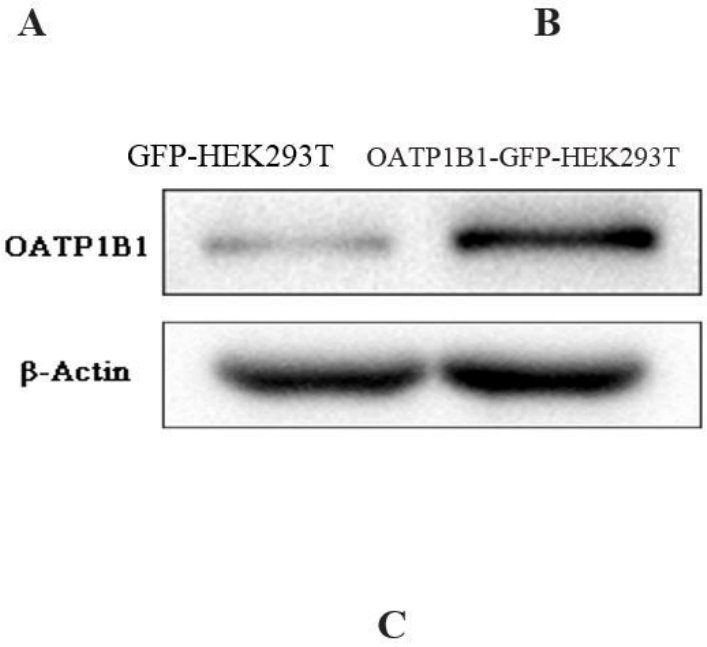


Figure 1

Successfully established OATP1B1-GFP-HEK239T cell model (A) in which OATP1B1 was highly expressed. Compared to the control GFP-HEK239T cells (B), OATP1B1 expression increased about 214% in the OATP1B1-GFP-HEK239T cells.Both, GFP-HEK239T cells and OATP1B1-GFP-HEK293T cells were cultured in regular DMEM with 10% FBS. OATP1B1expression in the GFP-HEK293 cells and HEK239T-OATP1B1 cells were detected by western blot(C), and showed significant difference between the two cell models. β-actin served as an internal control.

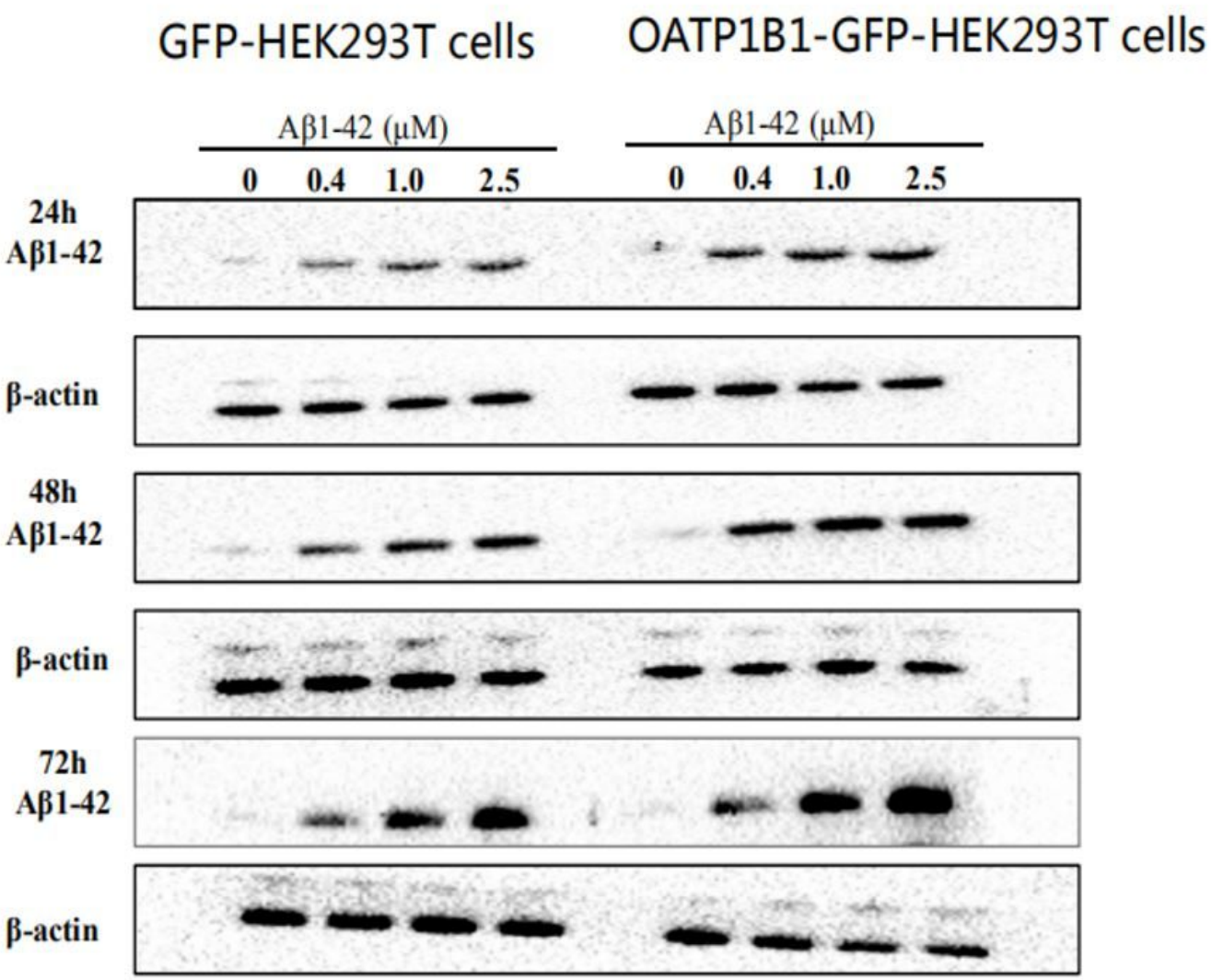


Figure 2

GFP-HEK239T cells and OATP1B1-GFP-HEK293T cellswere cultured in 6-well plates at 2.5 x 105 cells/well and after adherence, were treated withAβ1-42 (0, 0.4, 1, 2.5μM) for 24, 48, and 72 h. Following treatment, Aβ1-42 was determined in the cell lysates by western blotting (WB). Compared to the GFP-HEK239T cells group, the uptake of Aβ1-42 protein in the OATP1B1-GFP-HEK293T group increased with the increase in Aβ1-42 concentration. The increase was significant with the increase in incubation time. β-actin served as an internal control.

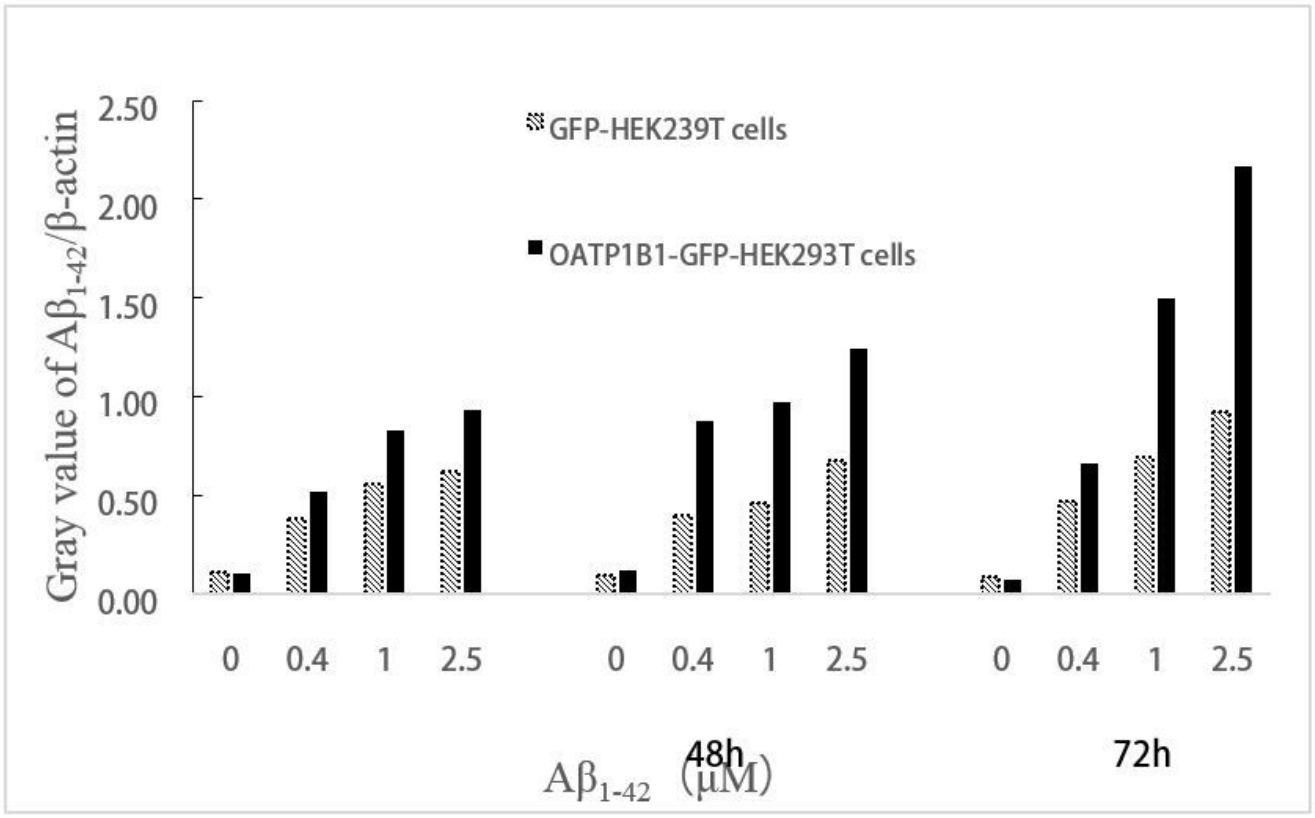


Figure 3

Western Blot bands were calculated and Aβ1-42/β-actin ratio value represented the indirect uptake of Aβ1-42 in the cells. The results showed that Aβ1-42uptake in GFP-HEK239T cells and OATP1B1-GFP-HEK293T cells was 0.11 vs 0.10, 0.38 vs 0.52, 0.56 vs 0.83, 0.62 vs 0.93 when the cells were treated with Aβ1-42 (0, 0.4, 1, 2.5 μM) for 24 hours, while they were 0.09 vs 0.12, 0.40 vs 0.87, 0.46 vs 0.97, 0.68 vs 1.24 for 48 hours, and 0.08 vs 0.07,0.47 vs 0.66,0.69 vs 1.49,0.92 vs 2.16 for 72 hours.

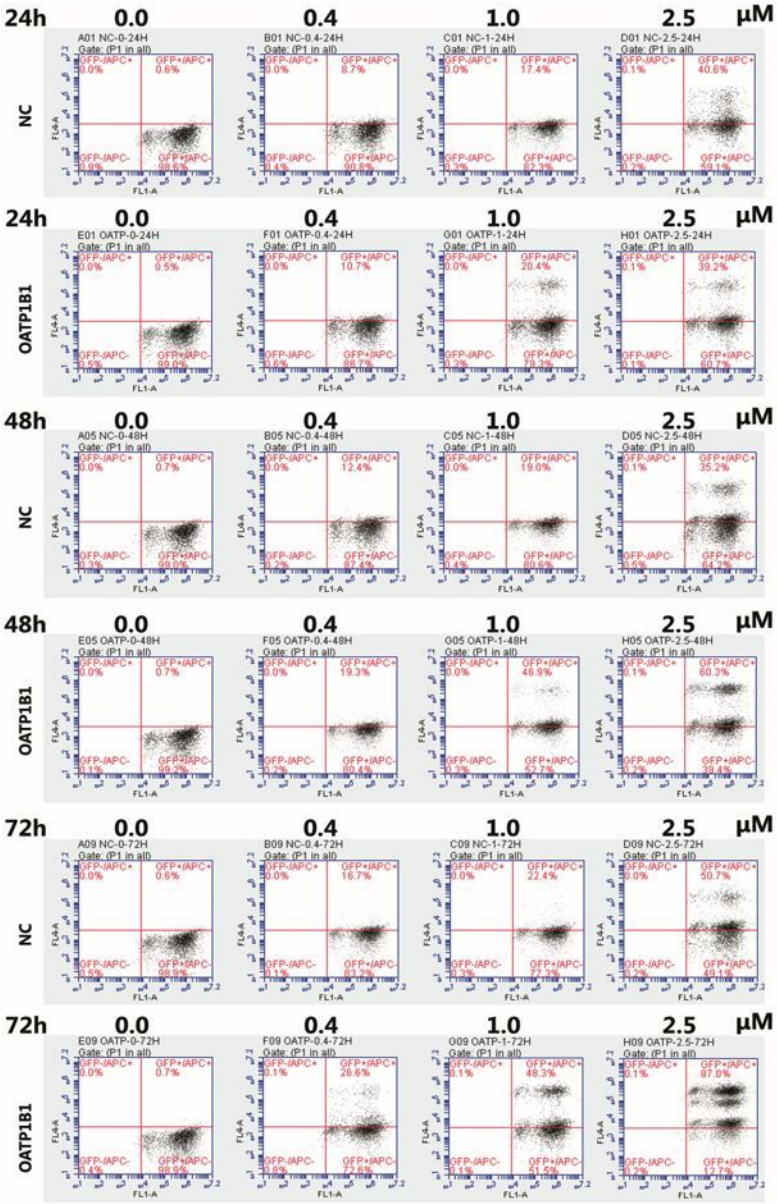


Figure 4

In the flow cytometry diagram, GFP fluorescence of cells was detected by transverse FL1A channel, and Aβ 1-42 Label fluorescence was detected by longitudinal FL4A channel. The double positive cells in the upper right quadrant were HEK293T cells successfully carrying GFP and ingesting Aβ1-42. By calculating the proportion of double positive cells in the upper right quadrant, the proportion of cells in each group that ingested Aβ1-42 was obtained (Figure 4 and Table 1). By calculating the fluorescence intensity of Aβ1-42 in the double positive cells in the upper right quadrant, the relative amount of A β1-42 ingested in each group of cells was obtained (shown in Figure 5 and table 2).NC:GFP-HEK239T cells;OATP1B1:OATP1B1-GFP-HEK293T cells.

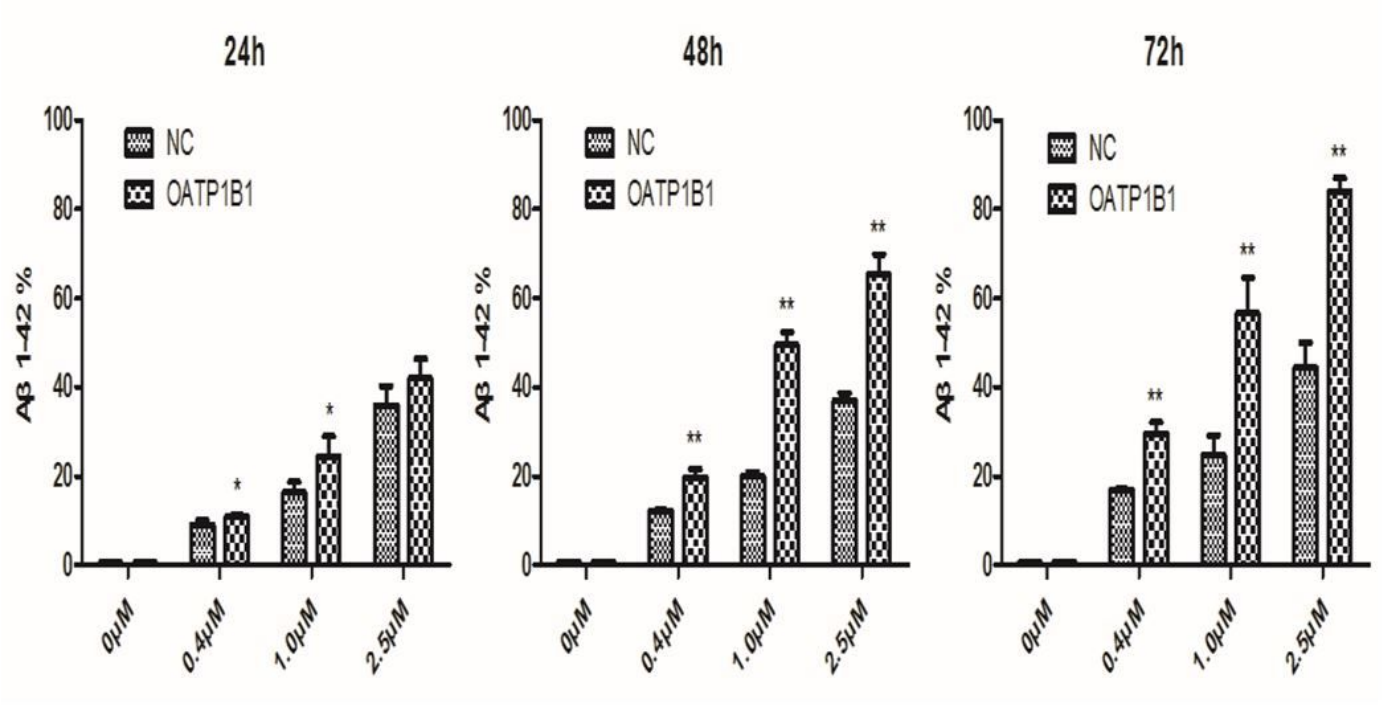


Figure 5

Flow cytometry showing the cellular uptake of Aβ 1-42 in GFP-HEK239T cells and OATP1B1-GFP-HEK293T cells following incubation with 0.4, 1, 2.5 μM Aβ (1-42) for 24, 48, 72 h, respectively. Uptake of Aβ 1-42 increased both in GFP-HEK239T cells and OATP1B1-GFP-HEK293T, and compared to GFP-HEK239T cells, the uptake of Aβ1-42 in OATP1B1-GFP-HEK293Tcells increased significantly with the increase in duration of incubation. Especially, when the incubation time was 72 h, the uptake of Aβ 1-42 in OATP1B1-HEK293T was almost twice as much as that in the HEK239T group. *indicates P<0.05; **indicates P<0.01. NC: GFP-HEK239T cells; OATP1B1:OATP1B1-GFP-HEK293T cells.

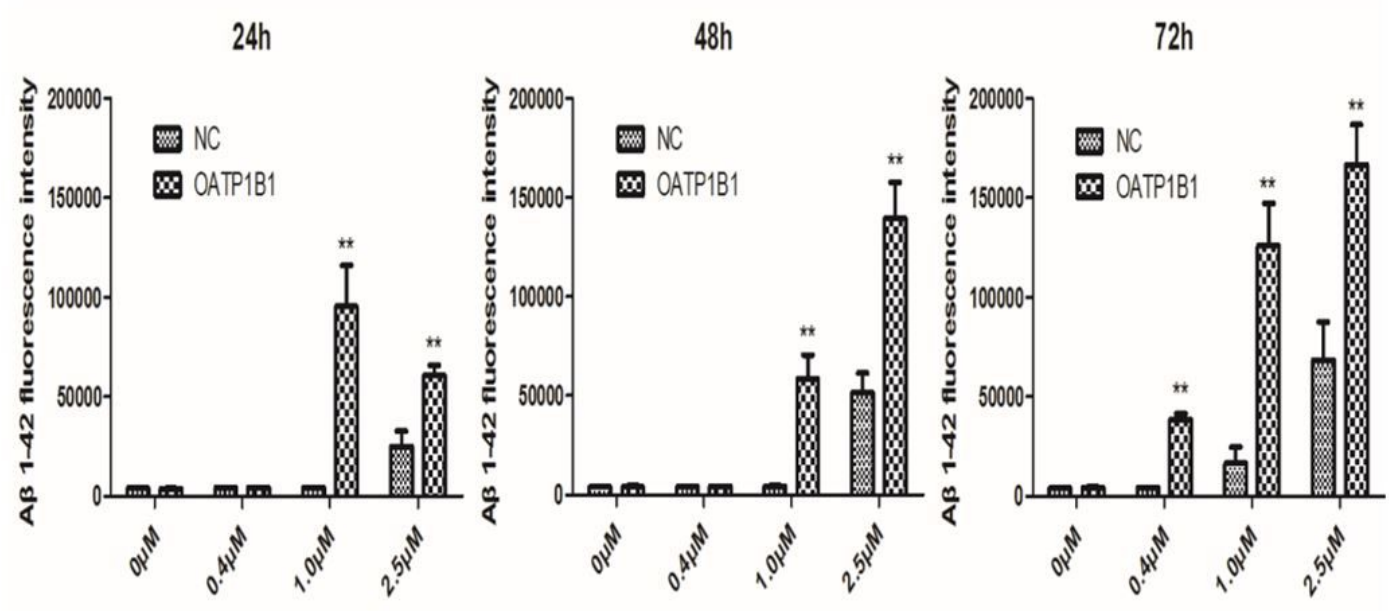


Figure 6

Fluorescence intensity of Aβ1-42 in the GFP-HEK239T cells and OATP1B1-GFP-HEK293T cells. The fluorescence intensity of Aβ 1-42 in OATP1B1-GFP-HEK293T cells was apparently higher than that in the GFP-HEK239T cells when the cells were treated with 1.0 and 2.5 μM Aβ1-42during the incubation time of 24-72 h. Especially, as the duration of incubation increased to 72 h, the fluorescence intensity of Aβ 1-42 in OATP1B1-GFP-HEK293T cells was significantly higher than that in the GFP-HEK239T cells in the concentration range of 0.4-2.5 μM. *indicates P<0.05; **indicates P<0.01. NC:GFP-HEK239T cells; OATP1B1:OATP1B1-GFP-HEK293T cells.

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