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Development of the alternative method for renal drug excretion using *Xenopus* oocyte expression system combined with a high throughput method, *OOCYTEXPRESS*®

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Abstract

Organic anion transporters (OATs) play an important role in the renal elimination of drugs. To date, we succeeded in the development of *in vitro* pharmacokinetics prediction system by establishing the cell lines stably-expressing several OATs. Although this system is suitable to evaluate the uptake of drugs, it seems not completely appropriate to examine their efflux. Here, we tried to develop a novel high-throughput method to evaluate the efflux function of drug transporters by using *Xenopus* oocytes expression system combined with a high throughput method, *OOCYTEXPRESS*®. In this study, we examined the efflux function of renal apical organic anion transporter NPT1 whose efflux function is still unknown. To evaluate the drug efflux, *in vitro* transcribed human NPT1 (hNPT1) cRNA was injected into oocytes. After three days incubation, we again injected the [^{14}C]*p*-aminohippuric acid (PAH) directly into the oocytes and measured the radioactivities of the outside buffer. hNPT1 cRNA-injected oocytes showed significantly higher count than control, indicating that hNPT1 can function as a drug efflux transporter. These results indicated that our method for renal drug excretion combined with the *OOCYTEXPRESS*® seems suitable for the evaluation of the drug efflux and is an alternative method for mammalian kidney slices.

Keywords: renal transporter, efflux, drug excretion, high throughput method, *OOCYTEXPRESS*®

Introduction

Xenopus oocyte expression system is traditional and familiar method for experiment. This is very useful technology, but at the same time there is a lot of pitfall, too. Merits are that (1) it is possible to get necessary data for drug development quickly, (2) it is easy to express various genes, (3) negative controls show low activity (low background), (4) it is possible to inject several cRNAs at the same time, (5) it is possible to reduce animal usage, and so on. Pitfalls are (1) low reproducibility because of *Xenopus* condition, (2) low throughput because of time consuming, (3) fluctuating gene expression rate, and so on. These pitfalls will be solved by *OOCYTEXPRESS*®, and we will suggest method to make the most of oocyte expression system. *OOCYTEXPRESS*® is innovated *Xenopus* oocytes assay service for the functional analysis of membrane protein like transporter, channel and so on. We have Standard Operating Procedures (SOP) at *Xenopus* breeding, oocytes selection, and Gene injection to get good oocytes. Solution of oocyte expression pitfalls in our SOP are (1) reduction of seasonal affect for oocyte maturation by controlling the frog living in low temperature and hormone injection to frogs at the appropriate period, (2) using oocyte selective manual with high quality to reduce individual variation, (3) using automated injection system to introduce constant amount of gene into large volume of oocytes. Using this device, we can control accurate injection with advanced CCD camera for visual and we can do quick injection around 96 oocytes/10 min. *OOCYTEXPRESS*® made us to get good oocytes to evaluate renal drug excretion. So we tried to analyze mechanism using following oocyte influx and efflux assay.

Materials and methods

Human urate transporter 1 (hURAT1) (Enomoto et al, 2002) and human sodium-dependent phosphate transporter type 1 (hNPT1) (Uchino et al, 2000) cRNAs were *in vitro* transcribed and injected into *Xenopus* oocytes by automatic injection machines. Transport function of hURAT1 and hNPT1 expressed

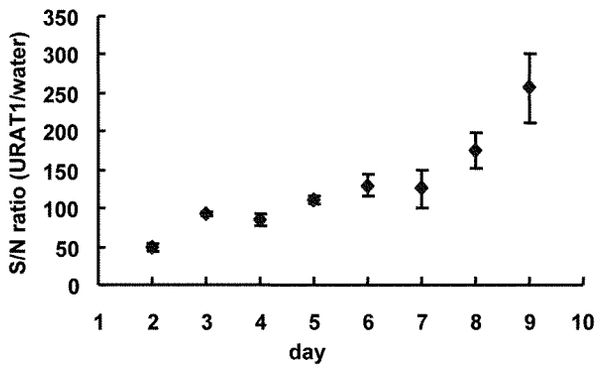

Fig. 1A. Influence of culture duration (2-9 days) after injection. Uptake activity showed [^{14}C] uric acid uptake ratio (hURAT1 cRNA injected oocytes / water injected oocytes). The uptake of [^{14}C] uric acid at the concentration of 30 µmol/L by water injected and URAT1 cRNA injected oocytes was measured for 60 min. Each point represents the mean ± S.E. from 10-12 oocytes.

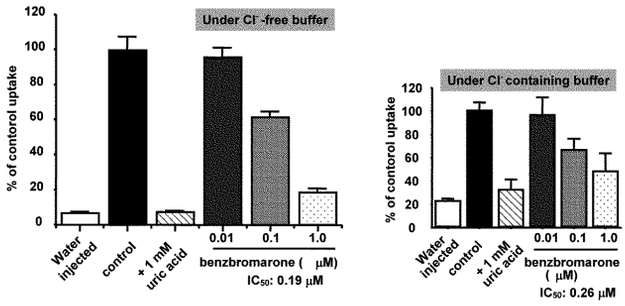

Fig. 1B. Inhibitory study of hURAT1.The uptake of 50 µmol/L [^{14}C] uric acid by water-injected or URAT1-cRNA-injected oocytes was measured in the presence or absence (control) of benzbromarone (0.01, 0.1, 1 µmol/L) for 60 min under Cl^- including buffer or not. Each point represents the mean ± S.E. from 9-10 ocytes.

in the oocytes was confirmed by measuring the uptake of radiolabelled compounds such as uric acid and PAH, respectively. To evaluate the drug efflux, we injected the radiolabelled compounds directly into the oocytes that expressed hNPT1 as reported previously (Jutabha et al, 2003), incubated oocytes in the buffer for 30, 60 min, and measured the radioactivities of the buffer using liquid scintillation spectrometry.

Results

We tried to evaluate the influx and efflux function of drugs and endogenous substances via transporters using oocytes expressing renal organic anion transporters prepared by *OOCYTEXPRESS*®. First we could obtain high transport activity of urate in a day-dependent manner using oocytes expressing hURAT1 after 2 to 9 days injection of its cRNA (Fig. 1A). After estimation of transport activity of urate in a day-dependent manner using the oocytes expressing hURAT1, we evaluated pharmacological effects of drug targeting hURAT1. We could demonstrate the pharmacological action of uricosuric agent benzbromarone by evaluating its IC_{50} of hURAT1-mediated urate transport (Fig. 1B). We tested oocyte quality by chemical injection. We did the preliminary experiments of inulin and mannitol for the confirmation of no leak after the injection procedure. It rarely detected the diffused inulin or mannitol to outside (Figs. 2A and 2B). After this preliminary experiment, we showed the usefulness of our system for efflux transporter function. So we did PAH injection into oocytes expressing hNPT1 for assessment of hNPT1-mediated PAH efflux. Before efflux assay, we tested hNPT1 function by influx of PAH. Furthermore we did inhibition of hNPT1-mediated PAH influx by uric acid, bumetanide, and salicylate (Fig. 3A). We got clear function of hNPT1, so we could demonstrate high efflux function of PAH via proposed renal efflux transporter of organic anions hNPT1 (Fig. 3B).

Discussion

We tested quality of oocytes derived from *OOCYTEXPRESS*® by various experiments. We were highly successful in typical transporter assay using uptake (influx) method. As a result, to evaluate membrane integrity of oocytes, mannitol (low-

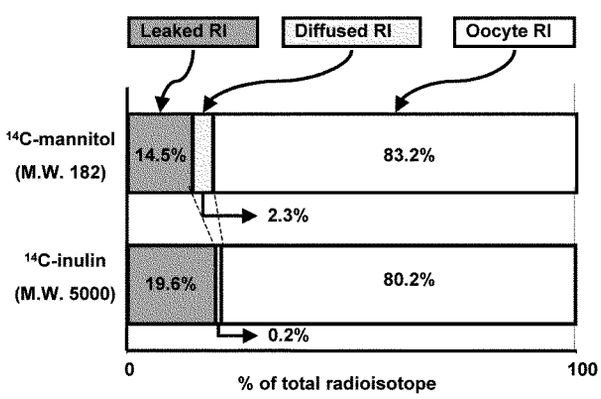

Fig. 2A. Ratio of loss by leakage and diffusion.

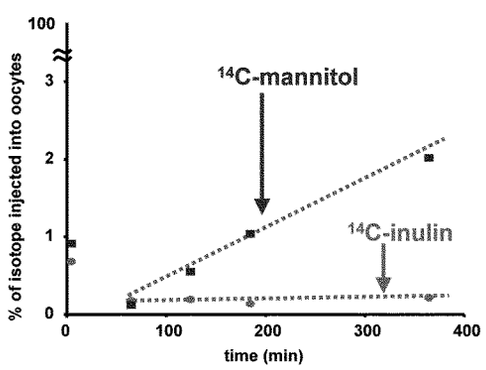

Fig. 2B. Diffused compound from oocytes incubated in buffer for 0-360 min.

Fig 2. Functional evaluation of efflux. The oocytes were microinjected with 32.2 nl of inulin and mannitol solution and incubated in buffer for 0-360 min.

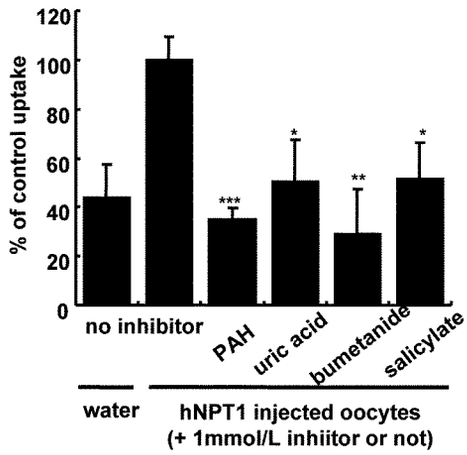

Fig. 3A. The uptake of 50 μmol/L [^{14}C] PAH by water injected or hNPT1 cRNA injected oocytes was measured in the presence or absence (control) of various inhibitors (1 mmol/L) for 60 min.

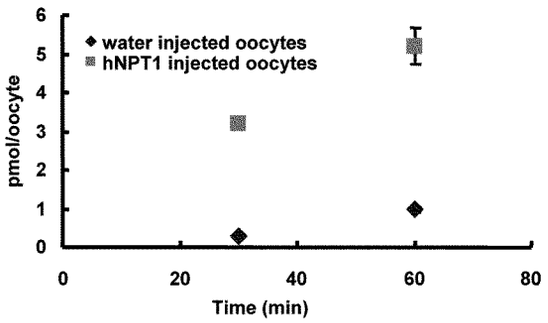

Fig. 3B. [^{14}C]PAH (0.1 mCi/ml) efflux from hNPT1 expressing oocytes and water injected oocytes. These oocytes were incubated in buffer for 30-60 min.

Fig. 3. Inhibitory and efflux study of hNPT1.

molecular weight) or inulin (high-molecular weight) was injected into oocyte and measured diffused compound. It rarely detected the diffused inulin or mannitol to outside because of good oocytes. Diffusion of mannitol increased in a time-dependent manner while that of inulin was constant during the measurement. Because the ratio of effluxed mannitol was limited to 2 % after 360 min from injection and was increased linearly, we concluded that effluxed mannitol was due to the simple diffusion and not from the leak from the oocyte damage by the injection procedure. Therefore, these oocytes occurring from *OOCYTEXPRESS*® are good condition for efflux study, and we could show hNPT1-mediated PAH efflux. It is very important for renal excretion of PAH to show hNPT1-mediated efflux.

OOCYTEXPRESS® made it possible to evaluate the influx and efflux function of drugs and endogenous substances via transporters using oocytes expressing renal organic anion transporters. In conclusion, our system will contribute to accelerate the procedure of drug development and to reduce the use of intact renal tissues or *in vivo* animal experiments.

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References

Enomoto, A., Kimura, H., Chairoungdua, A., Shigeta, Y., Jutabha, P., Cha, S.H., Hosoyamada, M., Takeda, M., Sekine, T., Igarashi, T., Matsuo, H., Kikuchi, Y., Oda, T., Ichida, K., Hosoya, T., Shimokata, K., Niwa, T., Kanai, Y., and Endou, H. (2002) Molecular identification of a renal urate-anion exchanger that regulates blood urate levels. *Nature*, 417, 447-452.

Jutabha, P., Kanai, Y., Hosoyamada, M., Chairoungdua, A., Kim, D.K., Iribe, Y., Babu, E., Kim J.Y., Anzai, N., Chatsudthipong, V., and Endou, H. (2003) Identification of a novel voltage-driven organic anion transporter present at apical membrane of renal proximal tubule. *The Journal of Biological and Chemistry*, 278, 27930-27938.

Uchino, H., Tamai, I., Yamashita, K., Minemoto, Y., Sai, Y., Yabuuchi, H., Miyamoto, K., Takeda, E., and Tsuji, A. (2000) p-aminohippuric acid transport at renal apical membrane mediated by human inorganic phosphate transporter NPT1. *Biochemical and Biophysical Research Communications*, 270, 254-259.