01B09-1

H⁺-COUPLED TRANSPORT OF ERYTHROMYCIN AT THE BLOOD-PLACENTA BARRIER IN RATS
Yoshimichi Sai, Akinori Takagi, Kaori Ochi, Tomohiro Nishimura, Noriko Kose and Emi Nakashima
Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan

Erythromycin (EM) is a macrolide antibiotics that is applicable to pregnant women who suffered chlamydial, syphilis and mycoplasma infections because of its less toxicity and limited placental transfer. Although EM is a known substrate for human and mouse OAT2, transport mechanism which limits the placental transfer has not been clarified yet. We have aimed to study the transport mechanism of EM at the blood-placenta barrier. The uptake of [¹⁴C]EM at the blood-placenta barrier was studied using a conditionally immortalized syncytiotrophoblast cell line, TR-TBT 18d-1. The [¹⁴C]EM uptake by TR-TBT 18d-1 cells took place in a Na⁺ or Cl⁻-independent manner. The [¹⁴C]EM uptake was slightly inhibited by p-aminohippurate and cimetidine but hardly affected by DHEAS and probenecid. The [¹⁴C]EM uptake was saturable showing a Km value of about 400 μM, which was more than ten-times larger than that by human OAT2. Macrolides such as Spiramycin, Azithromycin and Clarithromycin was inhibitory to the [¹⁴C]EM uptake. Josalycin did not inhibit the [¹⁴C]EM uptake, suggesting that the 14-16 membered ring and two sugars one of which had tertiary amine might required for the recognition by this system. Efflux transport by TR-TBT cells was assessed by examining effect of cyclosporin A, verapamil, quinidine and NaN₃ but none of them have affected the uptake. The [¹⁴C]EM uptake was pH-dependent. The saturable uptake was greatly increased according to increment of extracellular pH ranging from 6.4 and 8.4. The pH-dependency was further studied by using rat placental brush border membrane vesicles (BBMVs). The [¹⁴C]EM uptake by BBMVs was enhanced in the presence of outwardly directed H⁺ gradient. An over-shoot phenomenon was observed at pHₚₚ 8.4 and pHₚₚ 7.4 condition, suggesting involvement of an H⁺-coupled exchange mechanism. As Na⁺/H⁺ exchanger and H⁺-ATPase are expressed in the apical membrane of the syncytiotrophoblast, the H⁺ exchange may function to limit uptake of EM by the placenta.

01B09-2

TRANSPORT MECHANISM OF PURINE NUCLEOSIDES, ADENOSINE AND DIDANOSINE, ACROSS BRUSH-BORDER MEMBRANE IN RAT BLOOD-PLACENTA BARRIER
Tomohiro Nishimura, Kazuko Sato, Takuya Chishu, Noriko Kose, Yoshimichi Sai and Emi Nakashima
Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan

Blood-placenta barrier regulates transports of nutrients and xenobiotics between maternal and fetal blood. Nucleosides are pivotal source for nucleotides and nucleic acids, and are expected to be important in the growth of fetus. It has been shown that transfer of [³H]adenosine in the placenta is Na⁺-independent and is inhibited by nitrobenzylthioséoinosine (NBMPR), a selective equilibrative nucleoside transporter (ENT/SLC29) inhibitor. We have shown that adenosine would be taken up by ENT1 and ENT2 that are highly and poorly sensitive to NBMPR, respectively. We have also shown that didanosine, a nucleoside analog being used as a nucleoside reverse transcriptase inhibitor, was taken up by ENT2 using rat syncytiotrophoblast cell line, TR-TBT 18d-1 that our group previously established. Since it is expected that nucleosides are rapidly metabolized in cells, we were required to show the transport system(s) across brush-border membrane transported uncharged purine nucleosides. Therefore we employed rat placental brush-border membrane vesicles (BBMVs) that lack cytosolic metabolism. The purpose of the present study was to clarify the uptake mechanism of purine nucleosides and its analog from maternal side of rat syncytiotrophoblast using brush-border membrane vesicles prepared from rat placenta. [³H]Adenosine was rapidly taken up by BBMVs in a time-dependent manner. [³H]Adenosine uptake by BBMVs was clearly inhibited by excess concentration (2 mM) of unlabeled adenosine and was significantly but partly inhibited by 100 μM of NBMPR, being consistent with the previous results using TR-TBT 18d-1 cells. [³H]didanosine was also taken up by BBMVs and was inhibited by NBMPR. These results suggest that purine nucleoside transport system(s) across rat placenta brush-border membrane is mediated by the ENT family. The molecule responsible for the transport system(s) will be further characterized.