

การขนส่งโอคราทอกซินเอระดับเซลล์

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บทคัดย่อ

โอคราทอกซิน เอ (OTA) เป็นสารพิษที่พบการปนเปื้อนได้ในอาหารหลายประเภท OTA มีความเป็นต่อไต ตับ ระบบประสาท เป็นสารก่อวิรูปและเป็นพิษต่อระบบภูมิคุ้มกัน อวัยวะเป้าหมายหลักในการก่อพิษในมนุษย์คือไต การศึกษาการขนส่ง OTA ในระดับเซลล์เป็นสิ่งสำคัญที่จะสร้างความเข้าใจในกระบวนการดูดซึม การกระจาย และการขับออกของ OTA ในร่างกาย โปรตีนบนเยื่อหุ้มเซลล์ที่ใช้ในการขนส่ง OTA ได้แก่ Organic anion transporters (OATs), organic anion transporter polypeptide (OATPs), Breast cancer resistant protein (BCRP) และ multidrug resistant proteins (MRPs) โปรตีนขนส่ง OAT1, OAT3 และ OAT4 เป็นโปรตีนหลักที่ทำหน้าที่ในการดูดซึม OTA จากท่อไต ซึ่งมีหน้าที่การทำงานเช่นเดียวกับ novel human organic anion transporter NPT4 และ OATP1A2 ที่มีการแสดงออกอยู่ที่ท่อไต ส่วนอื่น OATP2B1 ที่มีการแสดงออกอยู่ที่ลำไส้เล็กอาจทำหน้าที่ในการดูดซึม OTA ที่ปนเปื้อนในอาหารผ่านทางช่องลำไส้เล็ก ในขณะเดียวกัน OATP1B1 และ OATP2B1 ที่มีการแสดงออกอยู่ที่เยื่อหุ้มเซลล์ตับไซโนซอยด์ สามารถเพิ่มการเมตาบอลิซึมและการขับสารพิษออกจากร่างกาย ผลโดยรวมของโปรตีน OATPs ที่มีต่อการขนส่ง OTA ในร่างกายนั้นขึ้นอยู่กับตำแหน่งของเนื้อเยื่อที่มีการแสดงออกของโปรตีนขนส่งนี้ ส่วน BCRP, MRP2 และ MRP4 เป็นโปรตีนขนส่งที่ทำหน้าที่ขับสารออกจากเซลล์ สามารถลดการสะสมของ OTA ในเซลล์และโดยรวมสามารถลดการเกิดพิษของ OTA ได้ การขนส่ง OTA ในระดับเซลล์นั้นพบว่าขึ้นอยู่กับ pH โดยการขนส่งจะเพิ่มมากขึ้นเมื่อ pH ลดลง ซึ่งเป็นผลมาจากการเปลี่ยนแปลงอัตราส่วนของไอออนิกโมเลกุล โดยสรุปความรู้ด้านการขนส่งระดับเซลล์ของ OTA ในเนื้อเยื่อชนิดต่างๆ สามารถนำไปประยุกต์ใช้ในการพัฒนาวิธีการป้องกันพิษของ OTA ได้

คำสำคัญ : โอคราทอกซิน เอ, OTA, ตัวขนส่ง, OATs, BCRP, MRPs

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Cellular Transporters for Ochratoxin A

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Abstract

Ochratoxin A (OTA) is a mycotoxin produced by several fungi species and found contaminated in foods. OTA exerts various toxicities including nephrotoxicity, hepatotoxicity, neurotoxicity, teratogenicity and immunotoxicity. The main target organ of OTA has been reported to be kidney. Study of molecular transport of OTA can be essential to develop profound understanding in OTA kinetics throughout the body. Membrane transporters found to be involved in OTA transport include organic anion transporters (OATs), organic transporter polypeptide (OATPs), breast cancer resistant protein (BCRP) and multidrug resistant proteins (MRPs). OAT1, OAT3 and OAT4 play a major role in cellular absorption of OTA in renal tubules. The same statement could be applied to a novel human organic anion transporter NPT4 and OATP1A2 that present at proximal tubular cells. OATP2B1 localized at intestinal cells might facilitate uptake of dietary OTA from the intestinal lumen. Meanwhile the expression of OATP1B1 and OATP2B1 at liver sinusoidal membrane could increase metabolism and excretion of OTA out of the cells. The net effect of OATP-mediated OTA transport in the body is dependent on its localization on each tissue. BCRP, MRP2 and MRP4 are efflux transporters and can reduce the net uptake of OTA into the cells and thus reduce OTA toxicity. The cellular transport of OTA is found to be pH dependent with increasing transport when pH decreases, which might be due to the changing in ionic portion of OTA. Overall, the knowledge on molecular transportation of OTA in various tissues could be applied for further development of strategic prevention of OTA-induced toxicity.

Keywords: Ochratoxin A, OTA, Transporters, OATs, BCRP, MRPs

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General characteristics of OTA and toxicities

Ochratoxin A (OTA) is a mycotoxin produced by several species of fungi including *Aspergillus* (mainly *A. ochraceus*, *A. niger*, and *A. Carbonarius*), *Penicillium* (mainly *P. verrucosum* and *P. Carbonarius*), *Petromyces*, and *Neopetromyces*. Among the group of ochratoxin, ochratoxin A is the most toxic and the most prevalent toxin found in agricultural commodities and natural environment. Foods that can be contaminated with OTA are pork, poultry, grains, wine, coffee, dried fruits¹. OTA is a pentaketide derived from dihydroisocoumarin family linked with β -phenylalanine. OTA is a weak acid, with 2 pKa values, 4.2 – 4.4 for the carboxyl group of the phenylalanine moiety and 7.0 – 7.3 for the phenolic hydroxyl group at the isocoumarin moiety, which means that under physiological pH (7.4), most of OTA presents as an organic anion².

Toxicological effects of OTA consist of nephrotoxicity, hepatotoxicity, neurotoxicity, teratogenicity and immunotoxicity. The major target organ of OTA in humans is kidney¹. The International Agency for Research on Cancer (IARC) classified the carcinogenic risk of OTA in group 2B (possibly carcinogenic to humans). Consumption of

OTA is evidently linked to the development of some renal diseases including Balkan endemic nephropathy and chronic interstitial nephropathy. However, the toxic mechanisms of OTA still remain uncertain³.

Membrane Transporters for Ochratoxin A: Isoforms and localization

OTA can be transported through cell membranes via passive diffusion or membrane transport proteins, which required energy – mainly ATP – to be functioned. There are several membrane transporters found to be associated with transepithelial transportation of OTA. Organic anion transporter (OAT, SLC22A) is a family of membrane transporter which comprises of a group of over 10 transmembrane proteins. OATs are localized in many tissues, including kidney, liver, choroid plexus, olfactory mucosa, brain, retina, and placenta. But, almost all of the isoforms are expressed in kidney. OATs have an essential role in regulating endogenous and exogenous organic anions in the body, including various drugs and toxins⁴.

Other membrane transporters are organic anion transporting polypeptides (OATPs, SLCO). OATPs comprise of six subfamilies (OATP1-6), with 11 isoforms in human. OATPs also transport organic

anions, but substrates of OATPs are mainly large hydrophobic organic anions compared with OATs substrates that usually smaller and more hydrophilic organic anions. OATPs are also expressed in many tissues including kidney⁵.

ATP-binding cassette transporters, one of the largest superfamily of membrane transport system are also involved with OTA transport. The ABC gene transporter can be divided into 7 subfamilies, with 48 isoforms in human being identified. These transporters regulate a wide variety of substrates including proteins, metal ions, sugars, hydrophobic compounds and metabolites. Mutations in ABC genes can contribute to several human genetic disorders for example cystic fibrosis, anemia and neurological disorder⁶.

Breast cancer resistant protein (BCRP/ABCG2) is one of the membrane transporter that belongs to ABC transporter superfamily. BCRP plays a role in transporting a wide variety of drugs and dietary toxins out of cells, thus can reduce systemic exposure of xenotoxins. BCRP are located at the apical side of kidney epithelia, intestine, placenta, and bile canalicular membrane of hepatocytes. Another important member of ABC transporter superfamily is multidrug resistance-associated protein (MRP/ABCC). ABCC family consists of

13 subfamily members (ABCC1 to ABCC13). MRPs are localized in many tissues including intestine, liver, brain capillaries, placenta and kidney. The transporters play an essential role in elimination of toxic substances, drugs and their phase II metabolites out of the body^{1, 7}.

As OTA can cause various toxic effects to many organs, especially kidney, consumption of OTA contaminated food can contribute to development of some renal diseases including Balkan endemic nephropathy and chronic interstitial nephropathy. So it is essential to have a profound understanding on the molecular mechanisms of OTA membrane transport, how it can accumulate in various organs especially kidney and which membrane transporters have a role in absorption or excretion of OTA. These understandings can be used further to develop strategies for prevention of OTA-induced toxicity.

Types of Transporters for Ochratoxin A

1. Organic Anion Transporters (OATs)

1.1 Basolateral side of proximal tubules

Investigation for the transport of OTA by rat kidney-specific organic anion transporter 1 (rOAT1) when expressing in *Xenopus laevis* oocytes cells showed that rOAT1 mediated uptake of OTA ($K_m = 2.1 \mu M$)⁸. Piroxicam and octanote, OAT1 inhibitors, have been shown to prevent nephrotoxicity from OTA, preventing

OTA nephrotoxicity by inhibition of OAT1. The effects of OTA on cell proliferation and cell viability were also investigated by using a cell line derived from the mouse kidney terminal proximal tubule (S3) transfected with OAT1 cDNA. Cell proliferation of S3 cells expressing OAT1 was suppressed when exposed to 2 and 10 μM OTA. This suppression was rescued by the co-addition of 1 mM p-aminohippurate, a known OAT1 inhibitor. It was, therefore, suggested that the nephrotoxicity of OTA involved with the OTA accumulation in proximal tubular cells via OAT1.

A further study demonstrated the OTA transport by human organic anion transporters (hOAT1 and hOAT3) by using the second segment of proximal tubule (S_2) cells from mice stably expressed hOAT1 and hOAT3 (S_2 hOAT1 and S_2 hOAT3)⁹. S_2 hOAT1 and S_2 hOAT3 exhibited a time- and dose-dependent, and a saturable increase in uptake of [^3H] - OTA. These OTA uptakes by hOAT1 and hOAT3 were inhibited by several substrates including PAH, probenecid, piroxicam, octanoate and citrinin. The inhibition is in a competitive manner ($K_i = 4.29 \sim 3080 \mu\text{M}$). The potency was as the following with hOAT1: probenecid > octanoate > PAH > piroxicam > citrinin; and for hOAT3 is probenecid > piroxicam > octanoate > citrinin > PAH. These

results indicated that both hOAT1 and hOAT3 mediate a high-affinity transport of OTA on the basolateral side of the proximal tubule, but the transport mediation are differently influenced by the substrates for the OATs.

1.2 Apical side of proximal tubules

The transport of OTA on the apical side of the proximal tubule was examined by using stably transfected mouse proximal tubule cells with hOAT4 (S_2 hOAT4)¹⁰. S_2 hOAT4 expressed hOAT4 protein in both the apical and basolateral sides of the cells and S_2 hOAT4 exhibited a time- and concentration-dependent, and a saturable increase in OTA uptake ($K_m = 22.9 \pm 2.44 \mu\text{M}$). The OTA uptakes were inhibited by probenecid, piroxicam, octanoate and citrinin – the substrates of OATs – in the competitive manner ($K_i = 44.4 \sim 336.4 \mu\text{M}$), indicating the mediation of hOAT4 on the high-affinity transport of OTA on the apical side of the proximal tubule.

Another transporter of OTA located at the apical side of renal proximal tubules has been identified as a novel human organic anion transporter hNPT4 (SLC17A3), classified as a type I sodium-phosphate cotransporter by using the *Xenopus* oocyte expression system¹¹. The results revealed that hNPT4 mediated time- and concentration-dependent uptake of OTA ($K_m = 802.8 \mu\text{M}$). The

transportation of OTA is pH and voltage dependent. The uptake of OTA rose when the pH decreased (pH ranges from 5.5 to 8.5). A noticeable finding is that the K_m of OTA for the efflux transporter hNPT4 was much higher than those for the uptake transporters hOAT1 and hOAT3 which may favor the accumulation of OTA in the tubular cell and lead to nephrotoxicity.

2. Organic Anion Transporting Polypeptides (OATPs)

OTA was first identified as a transport substrate for rat OATP1a1¹². Then various rat or mouse OATPs, including OATP1a1, OATP1a3v1, OATP1a3v2, and OATP1a, were subsequently reported to mediate OTA transport¹³. The investigation of cellular uptake of OTA by using HEK293 cells overexpressing cDNAs of the human uptake transporters OATP1A2, OATP1B1, OATP1B3, and OATP2B1 showed the significant increase of OTA cells overexpressing OATP1A2, OATP1B1, and OATP2B1 when compared with the mock cells and co-incubation with cyclosporin A markedly reduced the uptake. At pH 6.4 the uptake by OATP1A2 for OTA was most efficient followed by OATP2B1¹.

3. Multidrug Resistance Protein (MRP)

Roles of MRP on the transportation of OTA across intestinal epithelium cells was clearly demonstrated by using Caco-2

cell lines¹⁴. Several substrates of MRPs (indomethacin, genistein and probenecid, 1-chloro-2,4-dinitrobenzene) increased the apical to basolateral transport intracellular accumulation of OTA, indicating that OTA could be the substrate of MRP2 which localized at the apical pole of enterocytes. For other ATP-dependent membrane transporters such as P-gp, MRP2 and BCRP, secretion of OTA decreased and its absorption was found to be increased in the presence of BCRP- and MRP-inhibitors, indicating that BCRP and MRP2 play a role in secretion of OTA¹⁵. A recent study also showed that OTA was also a substrate of MRP4¹⁶. By conducting membrane vesicles transport studies prepared from HEK293 expressing hMRP4, the uptake of OTA by MRP4-expressing membrane vesicles was significantly higher than that of empty-vector membrane vesicles, suggesting that MRP4 also mediates OTA transportation.

4. Breast Cancer Resistant Protein (BCRP)

Breast Cancer Resistant Protein (BCRP) is another well-studied transporter for OTA. A study on transportation of OTA by using polarized MDCKII cells transduced with cDNAs of mBCRP and hBCRP showed the involvement of mBCRP in efflux of OTA from cells due to its ability to decrease the growth inhibition induced by OTA in MDCKII

cells¹. For the next experiment, investigating the transepithelial transport of OTA by mBCRP and hBCRP at pH 6.4 and 7.4. At pH 7.4, MDCKII parental and MDCKII-hBCRP cells showed insignificant apically directed transport of OTA. In the meanwhile, MDCKII-mBCRP cells showed some apically directed transport and was efficiently inhibited by Ko143, a specific BCRP inhibitor, indicating an involvement of BCRP in OTA transport.

At pH 6.4, the amount of OTA translocation was markedly increased with average translocation rates going up to 30 or even 100 pmol of OTA over 8 h. OTA was well transported by mBcrp and especially by hBCRP, with both transport processes being completely inhibited by Ko143. This data suggested that the transport of OTA was mediated by both mBCRP and hBCRP, and the activity increased under acidic condition.

pH - Dependent Membrane Transport for OTA

Generally, OTA membrane transport appeared to be dependent on pH. The lower the pH, the higher the membrane transportation. OTA has weak acidic properties, with 2 pKa values: 4.2 – 4.4 and 7.0 7.3². At physiological pH (7.4), around 50% of OTA will be dianionic, and the remainder mostly monoanionic, only a very small fraction is uncharged (~0.1%).

At pH 6.4, 80–90% of OTA will be monoanionic, a smaller fraction dianionic, and a small amount (about 1%) remains unchanged. So the difference in ionized form might interact with various membrane transporters differently.

Transport of OTA across cell membrane might occur via passive diffusion. There was 6- to 10-folds increase in apparent transmembrane movement of OTA in both parental MDCKII and HEK293 cells when shifting from pH 7.4 to 6.4, with lack of impact of Ko143 (a BCRP inhibitor) and Cyclosporin A (a broad – specificity OATP inhibitor). The capability of OTA in passive transmembrane diffusion might affected by pH. The increase in monoanionic or uncharged form results in increase of transmembrane diffusion. However, there is another possibility that OTA might be transported by some unidentified transporters that might work better at acidic pH¹. Noticeably, mildly acidic conditions readily occur in the lumen of proximal small intestine and renal proximal tubules, and in the space of diss. Therefore, pH effects are important in OTA disposition by these transporters.

OTA Transport in Various Organ Systems

The cellular uptake of OTA by OAT1, OAT3 and OAT4 at renal cells plays a significant role in OTA-induced

nephrotoxicity. All those transporters are presented in renal proximal tubule in which OAT1 and OAT3 are localized at basolateral surface while OAT4 localized at apical surface. Unlike OAT1, OAT3 is also expressed and function in the distal tubule. The cellular transportation of OTA by those transporters leads to OTA accumulation in renal cells and potentiate renal toxicity. OAT1 and OAT3 also presents at choroid plexus, where both has been found to transport the substrates from the CSF to the blood^{4, 17}. Moreover, OAT3 which are localized at brain capillary endothelium and retina also functions as an efflux transporter¹⁸. Therefore, the role of OATs in cellular mediation of OTA across various cell types needs to be further investigated.

The cellular uptake of OTA is also mediated by OATP1A2, OATP2B1 and, to a lesser extent, OATP1B1. OATP2B1 are localized at intestinal cells and might facilitate uptake of dietary OTA from the intestinal lumen, and thus increasing its body exposure. Meanwhile, the presence of OATP2B1 and OATP1B1 in the liver sinusoidal membrane could also eliminate OTA from blood into the liver for further metabolism and excretion into the bile, as the same by BCRP and MRP2. The process might increase liver exposure and toxicity. Overall, it reduces the body exposure through the blood. OATP1A2,

which expressed in the apical membrane of renal distal tubules might lead to reabsorption of OTA from pre-urine. In most cell types, OATPs likely enhance cellular uptake of OTA. But the overall effects of the transporters depend on its localization.

The presence of NPT4, BCRP, MRPs – the efflux transporters – can reduce the net uptake of OTA into the cells. For instance, BCRP and MRPs could reduce net uptake of dietary OTA from the intestinal lumen, and mediate excretion of OTA in liver hepatocytes into the bile. All those transporters also localized at apical side of proximal tubular cells, which can mediate OTA excretion into the urine. Although the role of BCRP in OTA secretion might not be apparent, sine the expression of BCRP on the apical membrane of human kidney proximal tubular cells is relatively low compared to that in the liver and small intestine¹⁹. The presence of these transporters in blood – tissue barrier sites such as the blood-brain, blood-testis and placental fetal-maternal barriers, could limit the OTA uptake into these cells. Thus, BCRP and MRP2 might help reducing the overall exposure of the body to OTA and limit its toxicity.

Conclusion

Various studies have been conducted in order to identify the membrane

transporters responsible for OTA transport. Until now, there are 5 groups of transporters that have been reported to regulate the transportation of OTA across the cells. Those transporters are OATs, OATPs, NPT4, MRPs and BCRP. OATs and OATPs are influx transporters. OAT1, OAT3 and OAT4 are localized at proximal and distal tubules, and play a major role in OTA uptake into the renal cells which lead to OTA-induced nephrotoxicity. For OATP transporters, OATP1A2 and OATP2B1 are expressed at enterocytes, which could lead to absorption of OTA from food. OATP1A2 also mediates OTA uptake at proximal tubule of the kidney. The presence of OATP1B1 and OATP2B1 at liver sinusoidal membrane could eliminate OTA from blood into the liver for further metabolism and excretion. BCRP, MRP2 and MRP4 are efflux transporters. They also localized at renal cells, enterocytes, and tissue barrier sites such as the blood-brain or blood-testis barrier. The presence of those transporters could reduce the net uptake of OTA into the cells and thus reducing OTA toxicity. NPT4 is another efflux transporter that expresses at proximal tubular cells and might reduce OTA toxicity. The transportation of OTA is pH dependent, with decreasing pH increasing the transportation. The reasons might be the increasing in monoanionic and uncharged

molecules in lower pH, which lead to different interaction with the membrane transporters and increase in passive diffusion. Overall, the knowledge on cellular transportation of OTA could increase understanding of how OTA aggravate toxicities, and thus help to develop strategies for prevention of OTA toxicity by preventing its absorption or enhancing its excretion.

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