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学位論文要旨

Therapeutic agents are essential to overcome diseases, but some drugs show serious problems in rare cases in their clinical treatment. The aim of this study was to clarify the mechanism of the pharmacokinetics of therapeutic agents as a direct considerable effort toward improving the therapeutic indices. Uptake of nafamostat mesilate (NM) across the basolateral and apical membrane of proximal tubular cells was examined *in vivo* and *in vitro*. The influx of NM across the basolateral membrane should cause the accumulation of NM in the kidney, resulting in the side effects of hyperkalemia. Grepafloxacin, a substrate of multidrug resistance associated proteins (MRPs), is reported to have a weak photosensitizing action, like other fluoroquinolones. I found that MRP1 was involved in the excretion of grepafloxacin in the skin. In addition, transdermal permeation of [^{3}H]flurbiprofen and [^{14}C]indomethacin was characterized to investigate the possible involvement of transporters in the transdermal permeation of two drugs. Effects of unlabeled compounds, medium pH and ATP-depressors were determined. Expression profile of xenobiotic transporters was screened in the skin of hairless mouse and human. I demonstrated the possible involvement of a certain types of nonlinear transport mechanism(s) in the transdermal permeation of flurbiprofen and indomethacin.

Transporter-mediated renal handling of nafamostat mesilate

Nafamostat mesilate (NM), an organic cationic compound, has been reported to accumulate in the kidney, which was proposed to block the urinary secretion of prostasin, resulting in the side effects of hyperkalemia and/or hyponatremia in rare cases. I attempted to clarify the mechanism of accumulation of NM in the kidney in chapter I. I demonstrated, by kidney uptake index method *in vivo*, that an organic

cation transport system(s) seems likely to be involved in NM uptake across the basolateral membrane of proximal tubular cells. Transcellular transport of NM across cultured monolayers of LLC-PK1 cells from the basolateral to the apical (B to A) and from the apical to the basolateral (A to B) directions was examined at different initial concentrations. The B to A transport was decreased significantly at higher initial concentration, suggesting the involvement of saturable transport system(s) in NM transport in the efflux direction. Using *Xenopus laevis* oocytes expressing systems, I demonstrated that NM was a substrate of organic cation transporters (OCTs) such as rOCT1, rOCT2 and hOCT2, which are expressed in the basolateral membrane of proximal tubular cells.

In the uptake of NM by brush-border membrane vesicles isolated from renal cortex of rats, a transport system was also involved in NM transport across the apical membrane of proximal tubules. No significant uptake of [^{14}C]NM was observed by HEK293 cells stably expressing hOCTN1 or hOCTN2 compared with mock cells, suggesting that NM was not a substrate of OCTNs. The transport of [^{3}H]daunomycin, a typical substrate of P-gp, in the B to A direction was greater than that in the A to B transport, but no directional transcellular transport of NM was observed, suggesting that NM was not a substrate of P-gp. The influx of NM from the basolateral membrane should result in the accumulation of NM in the kidney. The reason why NM accumulates in the kidney is likely to be that influx of NM at the basolateral membrane as a substrate of OCTs is highly efficient.

Transporter-mediated distribution in the skin

Skin is a dynamic organ having various defense mechanisms to protect the body from environmental factors. Grepafloxacin, a fluoroquinolone, is a substrate of MRP1, and is reported to have a weak photosensitizing action, like other fluoroquinolones. I determined tissue-to-plasma concentration ratio (Kp value) of grepafloxacin in mice, and found that Kp values of grepafloxacin in the skin of Mrp1 knockout mice (FVB/Mrp1(-/-)) was significantly higher than that of wild mice (FVB/Mrp1(+/+)), whereas no difference was observed in the profiles of the plasma concentration between FVB/Mrp1(-/-) and wild mice. Furthermore, I obtained the confocal images of 1-[2-amino-5-(2,7-dichloro-6-hydroxy-3-oxo-9-xanthenyl)phenoxy]-2-(2-amino-5-methylphenoxy)ethane-*N,N,N',N'*-tetraacetic acid (fluo 3), a fluorescent substrate of MRP1, in the skin slices *in vitro*. Accumulation of fluo 3 in the skin slices of hairless mice was increased by probenecid (2 mM) and FCCP (5 μM), but not by TEA (2 mM). Accumulation of fluo 3 in the skin slices of FVB/Mrp1(-/-) was significantly higher than

that of FVB/Mrp1(+/+) mice, suggesting that MRP1 is involved in the efflux of fluo 3 as an efflux pump in the skin. MRP1 is involved in the efflux of xenobiotics in the skin.

Characterization of transdermal transport of flurbiprofen and indomethacin

Transdermal route is frequently utilized for delivery of potent therapeutic agents with low molecular weight, and offers several advantages over conventional dosage forms. Log P values of drugs were used to predict the potential of their transdermal permeation, and lipophilicity of the enhancers was thought to play a major role in its activity. However, although the log P value of flurbiprofen (4.24) is similar to that of indomethacin (3.8), the transdermal penetration of flurbiprofen is much higher than that of indomethacin. I characterized and compared the transdermal permeation of [^{3}H]flurbiprofen and [^{14}C]indomethacin, by use of the Ussing-type chamber method. The permeability in an absorptive direction (P_{abs}) of [^{3}H]flurbiprofen was much higher than that of [^{14}C]indomethacin, whereas the permeability in a secretory direction (P_{sec}) of [^{3}H]flurbiprofen was almost similar to that of [^{14}C]indomethacin. Such phenomenon may not be explained by the passive diffusion of these compounds through multilayers of epidermis cells. Transdermal permeation of [^{3}H]flurbiprofen is increased with the increase in its initial concentration, suggesting that nonlinear mechanism(s) is involved in the penetration of this compound. The accumulation of both [^{3}H]flurbiprofen and [^{14}C]indomethacin in an absorptive direction remarkably increased (18.0-fold), whereas the P_{abs} of two compounds increased 50-fold and 22-fold, respectively, with the decrease in pH values from 7.4 to 5.0. Thus, the increase in P_{abs} of flurbiprofen cannot be described by the increase in accumulation of the two compounds, but a type of proton-dependent mechanism should be considered. In addition, coadministration of ATP-depressors significantly decreased the P_{abs} of [^{3}H]flurbiprofen, but slightly decreased that of [^{14}C]indomethacin, suggesting that ATP-dependent transport system(s) are involved in the absorption of [^{3}H]flurbiprofen, rather than that of [^{14}C]indomethacin. I found that the expression of xenobiotic transporters, such as MRP, OATP, OCTN and MCT family members in human skin, which was almost similar to that in the hairless mouse skin. Furthermore, I characterized the transport of two NSAIDs in human keratinocyte (HEK001 cells). In agreement with the results of transdermal permeation, effects of unlabeled compounds, medium pH and ATP-depressors were observed. In addition, high- and low-affinity transport was observed in the uptake of [^{3}H]flurbiprofen, but one component was observed in the uptake of [^{14}C]indomethacin, in agreement with that the affinity of transdermal transport of [^{3}H]flurbiprofen was higher than that of

[^{14}C]indomethacin. I demonstrated the possible involvement of a certain types of transporter-mediated mechanism(s) in the transdermal permeation of flurbiprofen and indomethacin.

学位論文審査結果の要旨

腎臓は薬物を排泄する主要な臓器であり、尿細管上皮細胞の形質膜に発現する薬物トランスポーター群が分泌や再吸収の分子機構として認識されつつある。一方、皮膚は他臓器に比べ細胞間液の容積が大きく血漿中と同レベルのアルブミンが存在するため、薬物の主要分布臓器の一つと考えられるが、皮膚での物質透過は主に角質層が形成する物理的バリアーに依存すると考えられてきた。本研究は、腎への蓄積により副作用である高カリウム血症が引き起こされると考えられるセリンプロテアーゼ阻害薬 nafamostat（NM）の腎移行、ニューキノロン薬 grepafloxacin の皮膚への分布、ならびに非ステロイド性抗炎症剤 flurbiprofen の皮膚透過機構に焦点をあて、両臓器における薬物の分布と排泄に関わる分子機構の解明を目指したものであり、以下の点が明らかとなった。

1）NM の腎移行には、尿細管血液側膜に発現する有機カチオントランスポーター OCT による効率的な取り込みと、管腔側膜上の有機カチオントランスポーター群による認識性の低さが関係すること。

2）皮膚には種々の薬物トランスポーター群が発現し、中でも multidrug resistance associated protein（MRP）1 は皮膚において排出ポンプとして機能し、基質である grepafloxacin の皮膚への分布に関与すること。

3）Flurbiprofen の経皮吸収および keratinocyte への取り込みには、濃度依存性、pH 依存性およびエネルギー依存性を示す何らかの輸送機構が関与すること。

以上の結果は、NM の腎移行と副作用発現機構の解明や今後の適正な薬物療法に有益な知見を与えるものと考えられた。また、皮膚における薬物輸送にトランスポーターが関与するという知見は本研究が初めてのものであり、経皮投与製剤の開発において有用な情報と考えられるため、本論文は博士（薬学）論文に値すると評価された。