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Pregnane X Receptor agonist Increases the Expression Levels of the Plasma Membrane Monoamine Transporter

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We evaluated the effect of the pregnane X receptor agonist, pregnenolone 16 alpha-carbonitrile (PCN) on the expression levels of plasma monoamine transporter (PMAT) in the intestine. Male C57/BL6 mice were divided into two 2 groups: mice in the PCN group (n=3) were administered PCN once a day for 4 days, while those in the control group (n=3) received the same volume of vehicle once a day for 4 days. After the mice were killed 24 h after administration of the last dose of PCN or vehicle, and the expression levels of PMAT in the intestine tissues were isolated and measured the expression level of PMAT using immunohistochemical and western blotting analyses. The expression level of PMAT expression levels in the small intestine increased after PCN treatment. These results suggest that the induction of PMAT may play a clinically significant role by increasing intestinal absorption of PMAT substrates such as metformin.

Introduction

Organic cation drugs are distributed in the body via a specific transport system consisting of organic cation transporters (OCTs) of the solute carrier 22 (SLC22) family and multidrug and toxin extrusion proteins (MATEs) from the SLC47 family.[1,2] Recently, Engel et al. reported a new polyspecific organic cation transporter known as the plasma membrane monoamine transporter (PMAT, SLC29A4), which is an Na^+-independent and membrane potential-sensitive transporter that transports monoamine neurotransmitters and neurotoxin 1-methyl-4-phenylpyridinium (MPP^+).[3] Functionally, PMAT shares its substrate and the specificity of its inhibitors with OCTs.[3-5] Metformin, an organic cation drug, is also transported by PMAT.[6] PMAT is expressed in the small intestine, kidney, heart, and brain.[6,7] Zhou et al. reported localization of PMAT in the mucosal epithelial layer of the human small intestine.[6] Considering that the Km value of metformin for PMAT, hOCT1, and hOCT2 is 1320, 1470, and 1380 μM, respectively,[6] metformin transportation by PMAT is comparable to that by OCTs. Since the expression level of PMAT in the small intestine is considerable, PMAT may play an important role in metformin absorption in the small intestine. Interestingly, our previous study has indicated that the absorption of metformin was increased in healthy volunteers after administration of rifampin, a pregnane X receptor (PXR) agonist.[8] Similarly, the PXR agonist pregnenolone 16 alpha-carbonitrile (PCN) has demonstrated potential effects of inducing some drug transporters in animal models. PCN increased the mRNA levels of OCTs, but not of the multidrug and toxic extrusion (MATE) transporter.[9,10] Therefore, we hypothesized that PCN increases PMAT's expression levels in the small intestine and may contribute to increased absorption of metformin. We determined the protein levels of PMAT in the presence and absence of PCN treatment in mice and compared PMAT expression levels.

ORIGINAL ARTICLE

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Materials and Methods

Animals

Six-week-old male C57/BL6 mice were used in this study. Experiments were performed in accordance with the guidelines and regulations of Yonsei University Medical College and approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Animals in the PCN group (n=3) were intraperitoneally (i.p.) administered a single dose of 75 mg/kg body weight (15 mg/ml in propylene glycol) once a day for 4 days. In contrast, animals in the control group (n=3) were injected with the same volume of vehicle once a day for 4 days. The mice were then sacrificed 24 h after administration of the last dose of PCN or vehicle.

Immunohistochemical analysis

To examine the tissue for morphological changes, we performed immunohistochemical staining on 4% formalin-fixed, paraffin-embedded tissue samples. Tissues sections attached to a saline-coated slide were incubated overnight at 37°C, deparaffinized in xylene (3×10 min), and rehydrated through a graded alcohol series (100%, 95%, 90%, 80%, 70%) diluted in water. The paraffinized sections were heated and then boiled (3×10 min) by microwaving in 0.01 M citrate buffer (pH 6.0) for antigen retrieval. Antibodies against PMAT protein (ab75615; 1:50; Abcam, UK) were applied and incubated at 4°C overnight. After 3 PBS washes, the sections were incubated in biotinylated link (LSAB2; Dako A/S, Glostrup, Denmark) for 20 min, and then washed with PBS 3 times. The peroxidase-binding site was detected by a diaminobenzidine reaction (DAB; DAKO A/S).

Western blot analysis

Western blotting was performed according to previously described methods using antibodies against PMAT (ab75615; 1:100; Abcam, UK).[11,12] Antibodies were used at the dilution recommended by the manufacturer. The samples in each group were pooled.

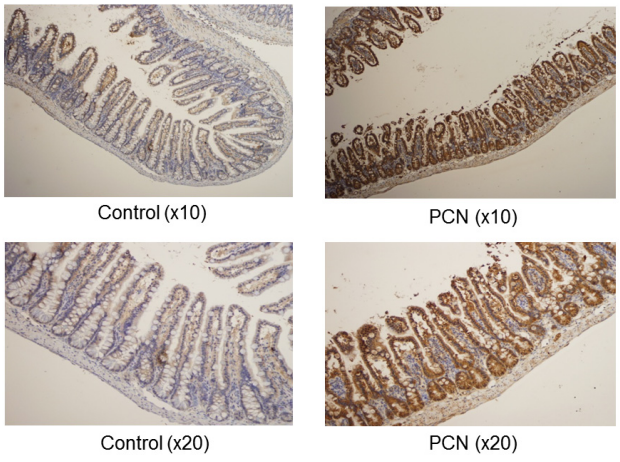

Figure 1. Effect of pregnenolone 16 alpha-carbonitrile (PCN) on PMAT expression in C57/BL6 mice. Representative immunohistochemical images for PMAT staining in the small intestine in the control (left) and PCN (right) animals.

Results

Histological and western blotting

Histological findings in the PCN group revealed a difference between the control group and PCN group of C57/BL6 mice. Figure 1 is a representative immunohistochemistry image for PMAT staining in the small intestine. The most obvious PCN-induced changes in the immunohistochemical findings were the increased expression levels of PMAT. The PCN administration group showed greater PMAT staining density than the control group located in the apical and basolateral membrane of epithelial cells in the small intestine. As shown in the western blot analysis in Figure 2, the protein expression levels of PMAT in the small intestine and liver also increased in PCN-treated mice.

Discussion

Consistent with the results of a previous report,[6] PMAT was expressed in the small intestine of the mice in our study. Our results demonstrated that PCN treatment increased the expression levels of PMAT in the small intestine. These result are in line with our previous clinical study that the PXR agonist rifampin enhanced the absorption of metformin in healthy volunteers.[8] PMAT is a known transporter of metformin in the small intestine.[6] Thus, the enhanced absorptive activity of metformin may be mediated by the PCN-induced increase in PMAT expression. The effect of the transactivator PXR has been well described in several studies on metformin transport. PXR has not been shown to affect the expression level of MATEs, but has demonstrated effects on OCTs.[9,10] However, the effect of PXR on PMAT expression has been unknown. To the best of our knowledge, this is the first study to report that a PXR agonist increases the expression levels of PMAT in the small intestine.

OCT1 and OCT3 are also localized in the intestine[13] but the expression level was much lower than liver. Although the relative intestinal or hepatic expression level of PMAT compared

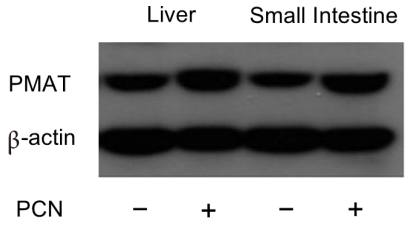

Figure 2. The effect of PCN on PMAT expression in the liver and small intestine as assessed using western blotting analyses (samples were pooled).

to OCT1 or OCT3 has been unknown in human, increased levels of PMAT may have contributed to the enhanced uptake of PMAT substrate such as metformin and possibly its effect. PMAT in other organs including liver and brain would be induced by PCN treatment. We also obtained liver samples and observed an increased expression in western blot analysis but immunohistochemistry finding was not definite (data not shown). Monoamine neurotransmitters (*e.g.* dopamine, serotonin) are known PMAT substrates and induced brain PMAT would. affect these neurotransmitters' activities.

There are some limitations in this study. First, no quantitative PMAT mRNA analysis was performed. PCN treatment is expected to increase PMAT mRNA by transcriptional up-regulation. However, it is rational to infer that the increased PMAT protein expression was mediated by increased transcription because PCN is a specific PXR agonist activating transcription. Second, other transactivators may contribute to the increased levels of PMAT. For example, the peroxisome proliferator agonist receptor-alpha and receptor-gamma have been shown to affect the transcriptional regulation of OCT1.[14] Moreover, despite the lack of available data on its effects, constitutive androstane receptor (CAR) might contribute to the transcription of this transporter.[15] In addition, the pharmacokinetics of PMAT substrates such as metformin before and after PCN treatment may contribute to the interpretation of our results. Third, the small number of samples and no statistical analysis may be not sufficient to warrant our conclusion, but the increased staining results in immunohistochemistry were consistent in all of the samples from different tissues. Finally, our results cannot be readily extrapolated to humans; it is necessary to measure the changes in PMAT expression levels in the human small intestine before and after rifampin treatment, which will require a more invasive endoscopic biopsy.

In summary, we found that the PXR agonist PCN increased the expression levels of PMAT in the small intestine. The results of this study imply that induction of PMAT may contribute to increased transport activity of PMAT substrates in the small intestine and probably enhance its absorption.

Acknowledgments

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Conflict of Interest

None of the authors has any conflicts of interest regarding this study.

References

1. Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. Biochem Pharmacol 2007;74:359-371.
2. Sugawara-Yokoo M, Urakami Y, Koyama H, Fujikura K, Masuda S, Saito H, et al. Differential localization of organic cation transporters rOCT1 and rOCT2 in the basolateral membrane of rat kidney proximal tubules. Histochem Cell Biol 2000;114:175-180.
3. Engel K, Zhou M, Wang J. Identification and characterization of a novel monoamine transporter in the human brain. J Biol Chem 2004; 279:50042-5049.
4. Ho HT, Pan Y, Cui Z, Duan H, Swaan PW, Wang J. Molecular analysis and structure-activity relationship modeling of the substrate/inhibitor interaction site of plasma membrane monoamine transporter. J Pharmacol Exp Ther 2011;339:376-385.
5. Engel K, Wang J. Interaction of organic cations with a newly identified plasma membrane monoamine transporter. Mol Pharmacol 2005;68:1397-1407.
6. Zhou M, Xia L, Wang J. Metformin transport by a newly cloned proton-stimulated organic cation transporter (plasma membrane monoamine transporter) expressed in human intestine. Drug Metab Dispos 2007;35:1956-1962.
7. Xia L, Zhou M, Kalhorn TF, Ho HT, Wang J. Podocyte-specific expression of organic cation transporter PMAT: implication in puromycin aminonucleoside nephrotoxicity. Am J Physiol Renal Physiol 2009;296:F1307-F1313.
8. Cho SK, Yoon JS, Lee MG, Lee DH, Lim LA, Park K, et al. Rifampin enhances the glucose-lowering effect of metformin and increases OCT1 mRNA levels in healthy participants. Clin Pharmacol Ther 2011;89:416-421.
9. Lickteig AJ, Cheng X, Augustine LM, Klaassen CD, Cherrington NJ. Tissue distribution, ontogeny and induction of the transporters Multidrug and toxin extrusion (MATE) 1 and MATE2 mRNA expression levels in mice. Life Sci 2008;83:59-64.
10. Maeda T, Oyabu M, Yotsumoto T, Higashi R, Nagata K, Yamazoe Y, et al. Effect of pregnane X receptor ligand on pharmacokinetics of substrates of organic cation transporter Oct1 in rats. Drug Metab Dispos 2007;35:1580-1586.
11. Kim W, Seong J, An JH, Oh HJ. Enhancement of tumor radioresponse by wortmannin in C3H/HeJ hepatocarcinoma. J Radiat Res 2007;48:187-195.
12. Seong J, Oh HJ, Kim J, An JH, Kim W. Identification of proteins that regulate radiation-induced apoptosis in murine tumors with wild type p53. J Radiat Res 2007;48:435-441.
13. Nies AT, Koepsell H, Winter S, Burk O, Klein K, Kerb R, et al. Expression of organic cation transporters OCT1 (SLC22A1) and OCT3 (SLC22A3) is affected by genetic factors and cholestasis in human liver. Hepatology 2009;50:1227-1240.
14. Nie W, Sweetser S, Rinella M, Green RM. Transcriptional regulation of murine Slc22a1 (Oct1) by peroxisome proliferator agonist receptor-alpha and -gamma. Am J Physiol Gastrointest Liver Physiol 2005;288:G207-G212.
15. Xu S, Sun AQ, Suchy FJ. A novel RARα/CAR-mediated mechanism for regulation of human organic solute transporter-β gene expression. Am J Physiol Gastrointest Liver Physiol 2014;306:G154-162.