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博士の専攻分野の名称	博　士（医　学）
学位記番号	医工農博４甲　第９１号
学位授与年月日	令和６年３月２２日
学位授与の要件	学位規則第４条第１項該当
専　　攻　　名	医学専攻
学位論文題名	Ethyl Caffeate Can Inhibit Aryl Hydrocarbon Receptor (AhR) Signaling and AhR-Mediated Potentiation of Mast Cell Activation (Ethyl Caffeate は Aryl Hydrocarbon Receptor（AhR）シグナル伝達と AhR を介したマスト細胞活性化の増強を阻害する)
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学位論文内容の要旨

Background

Ethyl caffeate (EC) is a natural phenolic compound that is present in several medicinal plants used to treat inflammatory disorders. However, its anti-inflammatory mechanisms are not fully understood. The aryl hydrocarbon receptor (AhR) is a chemical sensor for many common environmental contaminants (e.g., tetrachiorodibenzo-p-dioxin: TCDD), microbial metabolites (e.g., 1,4-dihydroxy-2-naphthoic acid: DHNA), and tryptophan metabolites (e.g., the tryptophan photoproduct FICZ). AhR is ubiquitously expressed in vertebrate cells, including mast cells. In the past decade, there has been increasing evidence that AhR signaling plays an important role in regulating innate and adaptive immune responses beyond xenobiotic metabolism. AhR controls IgE-mediated mast cell differentiation and activation and controls inflammation response.

Objective

This study aimed to determine whether EC can inhibit AhR signaling and AhR-dependent potentiation of IgE-mediated mast cell activation.

Method

In vitro, to evaluate the inhibitory effects of the AhR activity by EC, we performed SEAP assay using the HeXS34 reporter cells line; we examined gene expression, cytokine production and protein levels in the bone marrow-derived mast cells (BMMCs). We performed and compared the IgE-mediated mast cell degranulation between the RPMI 1640 and IMDM medium-cultured BMMCs, and degranulation in mucosal-type mast cells (MMCs).

In vivo, we examined gene expression in the small intestine and ears, and performed the passive cutaneous anaphylaxis (PCA) reaction.

Result

1. EC antagonizes AhR activation both in vitro and in vivo

We found that 10 μM EC alone did not have AhR agonistic activity, but instead, a pretreatment with 10 μM EC significantly inhibited the SEAP activity induced by FICZ or DHNA in HeXS34 reporter cells. FICZ- or DHNA-induced Cyp1A1 mRNA expression was consistently inhibited by 1 or 10 μM EC in BMMCs, and this inhibition also occurred following treatment with CH-223191, a well-established AhR antagonist. Twelve hours after FICZ exposure, AhR protein levels were

reduced in unstimulated BMMCs, as previously described; EC at 1 and 10 μM inhibited the FICZ-induced downregulation of AhR protein levels. In addition, AhR ligands alone were reported to stimulate mast cells to release IL-6. We consistently found that DHNA did induce IL-6 production by BMMCs, and EC inhibited this production at 10 μM.

Pretreatment of mice with EC (10 mg/kg, p.o.) or CH-223191 inhibited DHNA-induced CYP1A1 expressions in the small intestine.

2. EC Inhibits AhR-Mediated Potentiation of Mast Cell Activation

Several commercially available culture media contain AhR ligands. For example, IMDM contains high amounts of AhR ligands when compared to the RMPI 1640 medium. Consistent with previous findings that AhR ligands potentiate IgE-mediated degranulation in mast cells, we found that BMMCs cultured for 2 weeks in IMDM showed enhanced degranulation upon IgE stimulation compared to those cultured for 2 weeks in the RPMI 1640 medium, as assessed by β-hexosaminidase (β-hex) release. Notably, both EC at 10 μM and CH-223191 significantly inhibited the IgE-mediated degranulation in BMMCs cultured in the RPMI 1640 medium or IMDM, as assessed by the β-hex and CD63 expression. In addition, both EC and CH-223191 inhibited the degranulation in MMCs differentiated in the co-culture of BMMCs with notch ligand-expressing Chinese hamster ovary cells.

Oral administration of EC or CH-223191 24 h prior to the induction of the PCA reaction significantly decreased ear swelling, the quantity of Evans blue dye extracted from ear tissue, and the serum MCP-1 levels compared to the vehicle treatment. The reduction witnessed in the PCA reaction by EC or CH-223191 was associated with the suppression of constitutively expressed CYP1A1 in the skin.

Discussion

This study is the first to show that EC has the potential to antagonize AhR signaling. EC has been reported to have not only anti-inflammatory activity but also antioxidant, anti-malaria, and anti-carcinoma activity. Since AhR signaling plays pleiotropic roles in several biological activities that overlap with EC activities, the inhibition of AhR signaling by EC may underlie its diverse biological effects. This study also showed that IgE-mediated mast cell activation might occur in the context of background endogenous AhR activity in vivo, suggesting that the blockade of the AhR signaling by EC might benefit allergic diseases involving IgE and mast cells. Both EC and CH-223191 inhibited the IgE-mediated degranulation in BMMCs cultured in RPMI 1640 medium or IMDM containing AhR ligands; the current findings suggest that the presence of AhR ligands in a cell culture medium influences the mast cell activation levels as well as Th17 cell differentiation and dendritic cell maturation. Therefore, an optimal medium selection seems necessary when studying BMMCs or MMCs in vitro. Our data suggest that the PCA reaction may occur with a background of endogenous AhR activation in mouse skin. The skin contains various AhR ligands, including FICZ. In addition, AhR-deficient mice show severe abnormalities in keratinization and skin barrier function. Thus, it is likely that persistent background AhR activity in the skin modulates the PCA reaction. The most important question is how EC can inhibit AhR signaling. The combination of EC and CH-223191 did not enhance the suppressive effects of each individual reagent on the IMDM-dependent increase in degranulation in BMMCs, suggesting that EC and CH-223191 might share the same target. Further research is required to determine if EC binds to AhR and evaluate its binding affinity.

Conclusion

EC can inhibit the activation of AhR, which suggests a novel mechanism underlying the anti-inflammatory activity of EC. Additionally, EC could prevent or treat allergic diseases involving IgE and mast cells since intrinsic AhR activity may play an important role in shaping allergic inflammation.

論文審査結果の要旨

In the doctoral thesis, Mr. TAN has studied to determine whether Ethyl caffeate (EC) can inhibit the aryl hydrocarbon receptor (AhR) signaling and AhR-dependent potentiation of IgE-mediated mast cell activation. He found that 1) EC alone did not have AhR agonistic activity, but EC significantly inhibited the SEAP (secreted alkaline phosphatase) activity. 2) Cyp1A1 mRNA expression was consistently inhibited by EC in BMMCs, and this inhibition also occurred following treatment with CH-223191, a well-established AhR antagonist. 3) After FICZ exposure, AhR protein levels were reduced in unstimulated BMMCs, and EC inhibited the FICZ-induced downregulation of AhR protein levels. 4) DHNA induce IL-6 production by BMMCs, and EC inhibited this production. 5) Pretreatment of mice with EC or CH-223191 inhibited DHNA-induced CYP1A1 expressions in the small intestine. 6) EC and CH-223191 significantly inhibited the IgE-mediated degranulation in BMMCs as assessed by the β-hex and CD63 expression. 7) Both EC and CH-223191 inhibited the degranulation in MMCs differentiated in the co-culture of BMMCs. 8) Oral administration of EC or CH-223191 24 h prior to the induction of the PCA reaction significantly decreased ear swelling, and the reduction witnessed in the PCA reaction by EC or CH-223191 was associated with the suppression of constitutively expressed CYP1A1.

Based on these findings, he concluded that EC can inhibit the activation of AhR, which suggests a novel mechanism underlying the anti-inflammatory activity of EC. Additionally, EC could prevent or treat allergic diseases involving IgE and mast cells since intrinsic AhR activity may play an important role in shaping allergic inflammation.

Overall, the present doctoral thesis is a well-performed study. The manuscript is clearly written and data are well interpreted, taking into account the existing literature in a solid and honest manner. This study provides several crucial clinical message, therefore this thesis is an important one.