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Perfluorohexadecanoic acid increases paracellular permeability in endothelial cells through the activation of plasma kallikrein-kinin system

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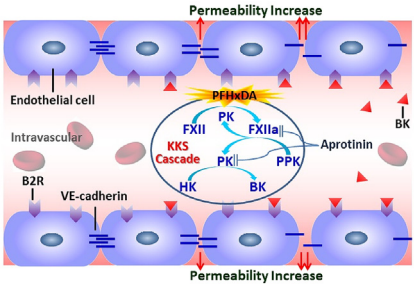
HIGHLIGHTS

- In contrast to PFOS and PFOA, PFHxDA could efficiently activate plasma KKS.
- PFHxDA-activated plasma increased paracellular permeability of HRECs through the degradation of adherens junctions.
- PFHxDA-involved effects on vascular permeability were mediated by KKS activation.

GRAPHICAL ABSTRACT



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ABSTRACT

Per- and polyfluoroalkyl substances (PFASs) are ubiquitous and high persistent in human blood, thus potentially inducing a myriad of deleterious consequences. Plasma kallikrein-kinin system (KKS), which physiologically regulates vascular permeability, is vulnerable to exogenous stimulators, like PFASs with long-chain alkyl backbone substituted by electronegative fluorine. The study on the interactions of PFASs with the KKS and the subsequent effects on vascular permeability would be helpful to illustrate how the chemicals penetrate the biological vascular barriers to reach different tissues. In present study, three representative PFASs, including perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexadecanoic acid (PFHxDA), were investigated for their effects on the activation of the KKS, paracellular permeability in human retina endothelial cells (HRECs) and integrity of the adherens junctions. In contrast to either PFOS or PFOA, PFHxDA efficiently triggered KKS activation in a concentration-dependent manner based on protease activity assays. The plasma activated by PFHxDA

Abbreviation: BK, Bradykinin; BSA, Bovine serum albumin; DMSO, Dimethyl sulfoxide; FXII, Hageman factor XII; FXIIa, Activated FXII; HK, High-molecular-weight kininogen; HRECs, Human retina endothelial cells; KKS, Kallikrein-kinin system; PFASs, Per- and polyfluoroalkyl substances; PFHxDA, Perfluorohexadecanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonic acid; PPK, Plasma prekallikrein; VE-cadherin, Vascular endothelial cadherin.

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significantly increased paracellular permeability of HRECs through the degradation of adherens junctions. As evidenced by the antagonistic effect of aprotinin, PFHxDA-involved effects on vascular permeability were mediated by KKS activation. The results herein firstly revealed the mechanistic pathway for PFHxDA induced effects on vascular endothelial cells. Regarding the possible structure-related activities of the chemicals, this finding would be of great help in the risk assessment of PFASs.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs), a large group of manufactured organofluorine compounds, contain fully or partially fluorinated alkyl backbone, and are mostly terminated with a polar functional group (Buck et al., 2011). The industrial usage of PFASs mainly includes fluorinated intermediates, fabrics coatings, fast-food contact materials, fire-fighting foams, hydraulic fluids, pesticides and so on (Calafat et al., 2007; Kissa, 1994). Some applications are closely related with people's daily life. During the production, usage and disposal of PFAS-containing products, this kind of chemicals are eventually destined to emerge in the environment. As PFASs break down very slowly, they are often characterized as persistent, thus posing widespread exposure risks for wildlife and humans (Zhao et al., 2012). Ubiquitous occurrence of PFASs has been reported in the blood of the general public (Liu et al., 2009; Olsen et al., 2003). The main pathway seems to be dietary intake (Berger et al., 2009; Domingo, 2012). Fluorochemical production workers and fishery employees were reported to have extremely high levels of PFASs, and the dominant species were perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) (Gao et al., 2015; Zhou et al., 2014). The retirees from fluorochemical factories were estimated to have a long serum elimination half-life for PFASs due to their negligible metabolism and renal clearance (Olsen et al., 2007; Seals et al., 2010). Accordingly, increasing concerns on health hazards from PFAS exposure are being gained, considering their global environmental contamination and widespread in blood circulation of human beings.

Despite of the important findings concerned with different toxicities, like hepatotoxicity, developmental toxicity, immunotoxicity, genotoxicity, and neurotoxicity induced by PFASs, (Kennedy et al., 2004; Lau et al., 2007), how exactly these chemicals penetrate the biological barriers and distribute in different organs, like liver, kidney, testis, and even brain (Kennedy et al., 2004; Lau et al., 2007) remains rather unknown as yet. It was proposed that PFASs could increase endothelial permeability through ROS-mediated actin filament remodeling, or tight junction disassembly triggered by phosphatidylinositol 3-kinase (PI3K)/Akt signaling activation (Qian et al., 2010; Wang et al., 2011). This biological process explains the direct stimulation of vascular endothelial cells by PFASs, but neglects the potential involvement of the endogenous biologically-active plasma components, which might encounter and react with the chemicals in the blood, thus posing effects on the vascular system.

The kallikrein-kinin system (KKS) is an important component of the plasma, and can mediate many physiological processes, wherein, the vascular permeability due to the release of inflammatory peptide, bradykinin (BK) from KKS activation, is one of the key responses (Fujisawa et al., 1995). The dysfunction of the vascular permeability could be sometimes clinically life-threatening (Davis Iii, 2005; Frank et al., 1976), while with respect to toxicological studies, it might facilitate the breakthrough of plasma toxins across the vascular barrier to reach the target tissues (Dejana et al., 2008; Komarova and Malik, 2010). The cascade activation of the KKS was characterized by the auto-activation of Hageman factor XII (FXII), subsequent cleavage of plasma prekallikrein (PPK), and final activation of high-molecular-weight kininogen (HK) (Feener et al., 2013). According to the characteristic auto-activation of FXII on negatively charged surfaces of nonphysiologic and physiologic elements (Schmaier, 2008), PFASs with long backbone alkyl chain substituted by electronegative fluorine could serve as FXII activators due to their extensive contact with the zymogen in the blood, and subsequently trigger the KKS cascade activation (Liu et al., 2017). Therefore, it would be of great interest to investigate the potential effects of PFASs on the KKS and the corresponding vascular permeability.

Although extensive toxicological researches (Cheng et al., 2013; Li et al., 2017) have been performed for the prevalent forms of PFASs, that is, PFOS and PFOA, which have been listed in the Stockholm Convention, the information on the potential biological effects caused by their congeners with relatively long chains are still far from sufficient. A few combined studies revealed that some long-chain PFASs could cause hepatotoxicity (Hirata-Koizumi et al., 2012; Takahashi et al., 2014), and the treatments with repeated doses could induce reproductive and developmental toxicities in rats (Hirata-Koizumi et al., 2015). The toxicities of PFASs were strictly correlated with their chemical structures, such as the carbon chain length (Hirata-Koizumi et al., 2015). Previous study has demonstrated that PFASs with longer carbon chain length, higher fluorine substitution degree and terminal acid group exhibited relatively higher activities in activating the KKS, wherein, perfluorohexadecanoic acid (PFHxDA) was found to be an efficient FXII activator (Liu et al., 2017). Therefore, it would be important to evaluate the potential effects of this specific long-carbon chain compound, instead of referencing the data from their well-known forms, i.e. PFOS, or PFOA.

In this study, PFHxDA, one representative PFAS congener with a long carbon chain, together with two prevalent forms of PFASs (PFOS and PFOA) were selected to screen their potentials in activating the KKS using protease activity assays. PFHxDA, as the potential activator of the KKS, was tested for its effect on the paracellular permeability of human retina endothelial cells (HRECs) through the activation of plasma KKS. The mechanism for the paracellular permeability increase was explored by testing the adherens junction integrity and aprotinin-based antagonistic experiments. The findings obtained in this study provided the new insight on the explanation of the compromised vascular integrity and target tissue permeation of long-chain PFASs.

2. Materials and methods

2.1. Ethical approval

All the experiments involving animals were conducted in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the ARRIVE guidelines. Animal experiments were