Journal of Medical and Biological Science Research
Vol. 2 (4), pp. 49-55, April, 2016
ISSN: 2449-1810
Full Length Research Paper
http://pearlresearchjournals.org/journals/jmbsr/index.html

Comparison of Vasocontractile Effects of Environmental Accumulating Material, Perfluorooctanesulfonate (PFOS) Among Isolated Rat Arteries

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Accepted 23 March 2016

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ABSTRACT

Perfluorooctanesulfonate (PFOS) and its perfluoro-analogues (PFAs) have been widely used in industrial application. The environmental persistence of PFAs has resulted in global occurrence of these substances. In the present study, vasocontractile effects of PFOS on isolated Wistar rats arterial rings were compared. Cumulative concentration response curves for PFOS (1 to 100μM) were obtained in thoracic aorta (TA), common carotid artery (CA), femoral artery (FA), pulmonary artery (PA), renal artery (RA) and supra-mesenteric artery (SMA) ring preparations. For structure-activity relationship study, PFOS was compared with perfluorooctanoic acid (PFOA), octanesulfonate (OS) or octanoic acid (OA). The most sensitive region was CA and 10 μM of PFOS showed significant contraction. The maximum contraction for PFOS on CA was larger than that of noradrenaline. Small contraction was also observed in RA, TA and SMA preparations, but not in FA and PA preparations at 100 μM of PFOS. Concerning structure-activity relationship, PFOS was the most potent compared with PFOA, OS or OA in CA preparation, suggesting the importance of carbon-fluoride structure as well as sulfonate. Present results indicated the possible toxicity of PFOS as an environmental contaminant and further studies on pollution of PFAs in the environment and biological effects of them are necessary.

Key words: Carotid artery, PFOS, Rat, Structure-activity relationship and Toxicity.

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INTRODUCTION

Perfluorooctanesulfonate (PFOS; Figure 1A Pottasium salt) and its perfluoro-analogues (PFAs) are characterized by a fully fluorinated hydrophobic linear carbon chain attached to various hydrophilic heads. The C-F bond is particularly strong, and resistant to various modes of degradation, including reaction with acids and bases, oxidation, and reduction (Kissa, 2001). Because of their unique physiochemical characteristics such as chemical (strong lipophobic and hydrophobic) properties and thermal stability as well as surface active properties which account for their ability to make materials stain, oil, and water resistant (Parsons et al., 2008; Suja et al., 2009). These compounds have been widely used in industrial, commercial household applications since 1950s (Renner, 2001; Jensen and Leffers, 2008) such as defogger of car window and Incombustible treatment. The widespread applications and environmental persistence of PFAs has resulted in global occurrence of these substances in water, sediments, sludge and air (Giesy and Kannan, 2001; Taniyasu et al., 2003; Martin et al., 2004; Higgins et al., 2005; Goosey and Harrad, 2012). The highest concentration in groundwater was 29 μM (that is 14600 μg/l ; Schultz et al., 2004) and that in sewage sludge was 53.83 μg/g (Guo et al., 2008). Their bioaccumulation in various wildlife species was reported (Giesy and Kannan, 2001; Kannan et al., 2002; Martin et al., 2004; Butenhoff et al., 2006; Suja et al., 2009; Houde et al., 2011).

The highest PFOS concentration reported in a fish blood from Lake Biwa in Japan was 1.6 μM (Taniyasu et al.,

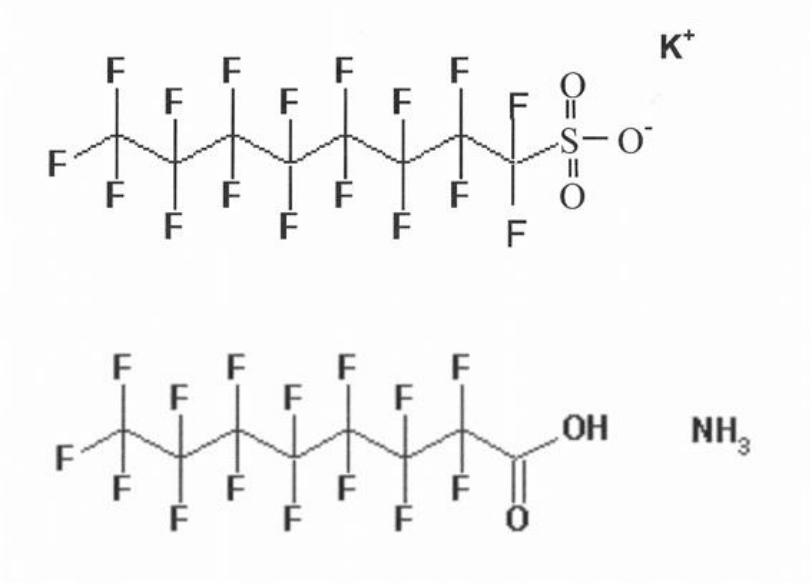

Figure 1. Figure 1A (Top) Structure of PFOS. **Figure 1B** (Bottom). Structure of PFOA.

2003). An increasing number of studies report high levels of PFOS in human samples such as blood, tissues, and breast milk (Houde et al., 2006; Calafat et al., 2007; Vestergren and Cousins, 2009). A maximal level of PFOS (3.49 µg/mL=7µM) was found in the serum of retired worker (Olsen et al., 2007). These results led to the increasing concerns about the potential detrimental effects of PFOS on human health and the environment. At Conference of the parties, held in May 2009 in Geneva, PFOS and its salts are being listed in the annexes of the Stockholm Convention on Persistent Organic Pollutants, a global treaty aiming to protect human health and the environment from persistent organic pollutants. Concerning bioactivities of PFAs, it has been considered to be relatively safety. For example, Xia et al. (2011) applied 2.0 mg/kg/day for 20 days to whole body during gestation and find mitochondrial injury in heart of dame, but not by 0.6 mg/kg/day. There was no information on blood or tissue PFOS concentration in the experiment, however, 40 mg/kg as total should be very high dose. Recently the effects of 0.2 µM of PFOS on mRNA and protein in mouse embryonic stem cell culture was reported (Xu et al., 2013). An objective of the present study was to compare the vasocontractile effect of PFOS on isolated Wistar rats arterial ring preparations to clarify the toxicity of PFOS on tissue level.

MATERIALS AND METHODS

Ten of Wistar strain male rats at 8 weeks of age were purchased from a commercial supplier (Shizuoka Experimental Animals, Inc., Shizuoka, Japan) and habituated for 2 weeks in the animal room before starting the experiment. Rats were euthanized by carbon dioxide. The changes of tension of isolated arterial ring preparations were measured by the methods previously described by Kawakoshi et al. (2004) with minor modification. TA, CA, FA, PA, RA and SMA were excised rapidly, the adhering fat and connective tissues trimmed off, and cut carefully into ring preparation 3 to 4 mm in width. The rings were fixed vertically in a 3 mL cuvette containing Krebs-Henseleit solution of the following composition (mmol/L): NaCl (118.4); KCl (4.7); $CaCl_2$ (2.2); KH_2PO_4 (1.2); $MgSO_4$ (1.2); $NaHCO_3$ (25.0); and dextrose (5.6). The resting tensions were 0.4 g for RA, 0.6 g for SMA and FA, 0.8 g for CA, 1.0 g for PA and 1.2 g for TA, respectively. The ring preparation was connected to the lever of a force-displacement transducer (Nihonkoden Kogyo Co, Ltd, Tokyo) and the change in isometric tension was recorded. The solution was aerated with a gas mixture of 95% O_2 and 5% CO_2 and maintained at 37±0.5°C and pH 7.4. All preparations were allowed to equilibrate for 120 min before initiating any experiment. The bathing fluid was exchanged every 20 min during the equilibration period. Rings were first contracted by 1 µM noradrenaline (NA) and washed to basic tension. Then, cumulative concentration response curves for PFOS (1 to 100 µM) were obtained in ring preparations.

The applied concentration range was equivalent to the maximum human serum concentration and less than those used for whole body studies. For structure-activity relationship study, PFOS activity on CA preparation was compared with perfluorooctanoic acid ammonium salt (PFOA; Figure 1B), octanesulfonate (OS) or octanoic acid (OA). Furthermore, comparison of responses to PFOS and PFOA was done in TA preparation. For endothelium removal, inside of ring preparation was rubbed gently with

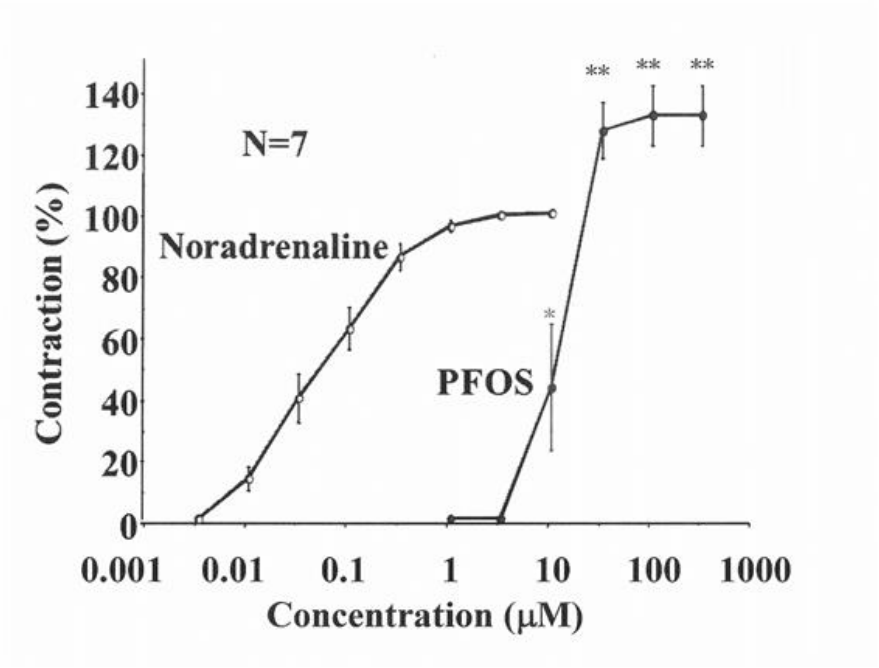

Figure 2. Comparison of vasoconstriction by PFOS and Noradrenaline in rat common carotid artery. Vertical bars indicate standard error. * $p < 0.05$ vs 3 μM. ** $p < 0.01$ vs 3μM.

stainless wire. Animal care and experimental procedures were approved by the Animal Research Committee of Shimane University and conducted according to the Regulations for Animal Experimentation at Shimane University. PFOS was purchased from TCI (Tokyo, Japan). Noradrenaline was purchased from Daiichisankyo (Tokyo, Japan). Other chemicals of analytical reagent grade were purchased from Wako Chemicals (Tokyo, Japan).

RESULTS AND DISCUSSION

The most sensitive region was CA and 10 μM of PFOS showed significant contraction (Figure 2). The contraction for PFOS was developed slowly and the tension kept stably. After washing, the tension returned to the basic level. The maximum contraction for PFOS on CA was larger than that of NA. Regional difference on vasoconstriction effects of PFOS was compared (Figure 3). The most sensitive response was observed in CA. Small contraction was also observed in RA, TA and SMA preparations, but not in FA and PA preparations at 100 μM of PFOS. Concerning structure-activity relationship, PFOS was the most potent compared with PFOA, OS or OA in CA preparation (Figure 4 left). PFOS was more potent compared with PFOA in TA preparation (Figure 4 right). No significant effect was observed with the remove of endothelium on PFOS-induced contraction in CA preparation (Figure 5).

The maximum contraction for PFOS on CA was larger than that of NA and 10 μM of PFOS showed significant contraction. Concerning toxic effects of PFOS, Xia et al. (2011) applied 2.0 mg/kg/day for 20 days during gestation and find mitochondrial injury in heart of dame, but not by 0.6 mg/kg/day. They suggested the possibility of the developmental toxicity, but it seems to be very high dose compared with natural accumulation of PFOS. Kim et al. (2011) indicated hepatic toxicity of PFOS, but, they reported non-observed-adverse effect level of PFOS was 1.25 mg/kg/day for 28days. In vitro study, Zhang et al. (2009) reported change of binding ratio of vitamin B2 to serum albumin by PFOS in mM order. Hu and Hu (2009) reported the effects of PFOS on antioxidative systems of Hep G2 cells in range from 50 to 200 μM. Xu et al. (2013) reported the effects of 0.2 μM of PFOS on Nanog mRNA and protein in mouse embryonic stem cell culture. Du et al. (2014) reported embryonic peripheral blood cell damage in Zebra fish by PFOS at 400 μg／L(8μM). Thus, embryonic damages may observed in μM or 0.1 μM order. Concerning occurrence of PFOS, the maximum concentration in mink liver was 0.0595 μg/g (Kannan et al., 2005) and that in polar bear liver was 4 μg /g (Martin et al., 2004).

The highest PFOS concentration reported in a fish blood from Lake Biwa in Japan was 1.6 μM (834 μg/l) and that in a fish liver in Okinawa was 7.9 μg/g (Taniyasu et al., 2003). A maximal level of PFOS (3.49 μg/mL~7 μM) was found in the serum of retired worker (Olsen et al., 2007). The concentration of significant contraction by PFOS in CA of rats was nearly equivalent to the maximum contamination

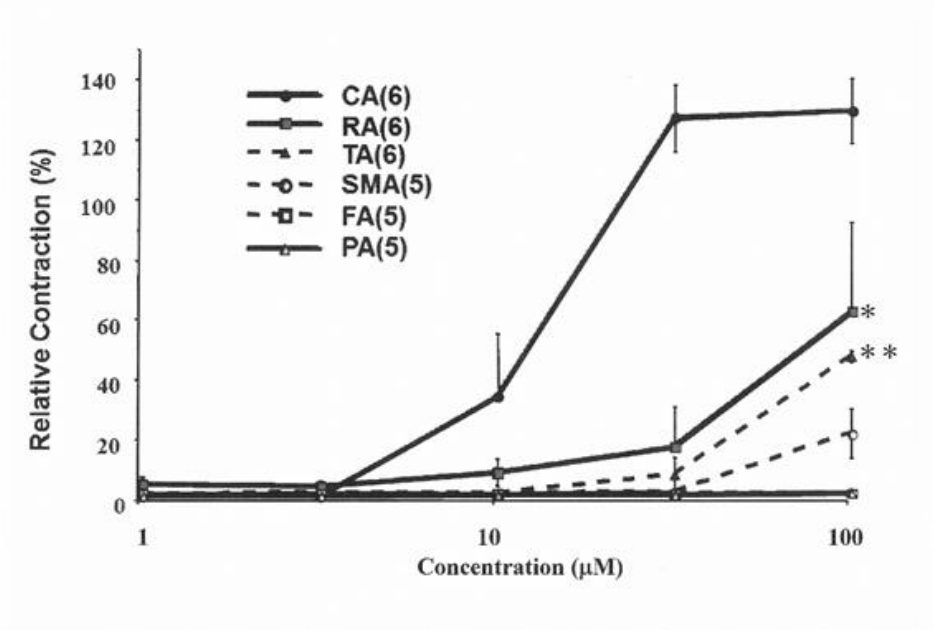

Figure 3. Regional difference on vasoconstriction effects of PFOS. Vasoconstriction effects of PFOS were compared among TA, CA, FA, PA, RA and SMA ring preparations. The number in parentheses indicates the number of preparation. Vertical bars indicate standard error. The most sensitive response was observed in CA. * $p < 0.05$ vs 3 μM. ** $p < 0.01$ vs 3 μM.

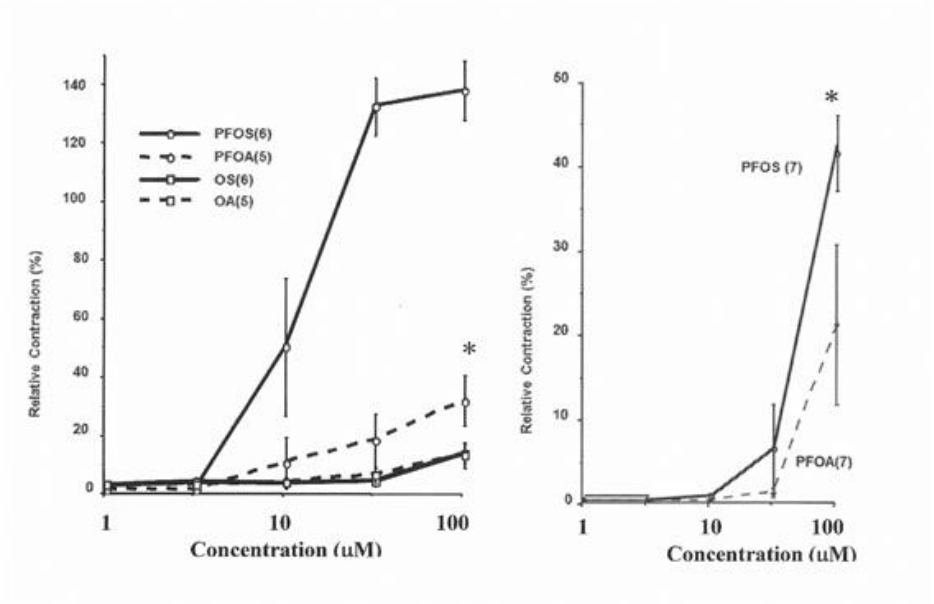

Figure 4. Structure-activity Relationship in carotid artery preparation (Left) and thoracic aorta preparation (Right). The number in parentheses indicates the number of preparation. Vertical bars indicate standard error. PFOS was the most potent compared with PFOA, OS or OA. * $p < 0.05$ vs 3 μM.

reported in human and wild animals. Small contraction was also observed in RA, TA and SMA preparations, but not in FA and PA preparations at 100 μM of PFOS. Clear regional difference was observed and the present result may suggest PFOS effect on brain circulation. Unfortunately we have no information why it is very active only on carotid artery. Further study is necessary to find the action mechanism of PFOS on vasculature.

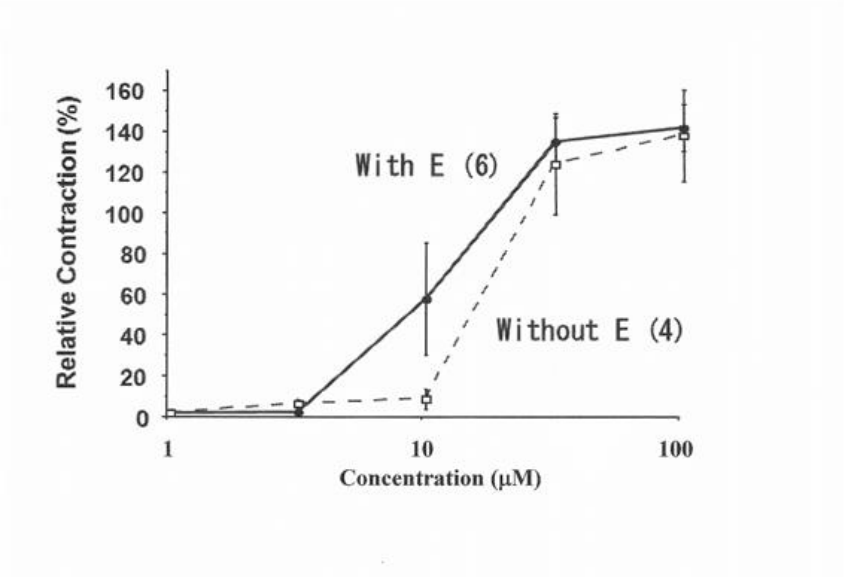

Figure 5. Effect of the remove of endothelium (E) on PFOS-induced contraction in carotid artery preparation. The number in parentheses indicates the number of preparation. Vertical bars indicate standard error.

Concerning structure-activity relationship, PFOS was the most potent compared with PFOA, OS or OA in CA preparation. In PFOA, only hydrophilic attachment was different and it showed week contractile effects. Sulfonate as hydrophilic attachment affected stronger than carboxyl base. The structure of PFOS and OS was the same expect C-F bonds was replaced to C-H bounds, however, no vasocontractile effects was observed by OS. These suggested the importance of carbon-fluoride structure as well as sulfonate. Endothelium may not be included in PFOS contraction.

CONCLUSION

Present results indicated the possible toxicity of PFOS as an environmental contaminant and further studies on pollution of PFAs in the environment and toxical effects of them are necessary.

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