学 位 論 文 の 要 旨

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学 位 論 文 名　Comparison of Vasocontractile Effects of Environmental Accumulating Material, Perfluorooctanesulfonate (PFOS) Among Isolated Rat Arteries

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論 文 内 容 の 要 旨

INTRODUCTION

Among a large number of man-made chemicals, perfluorooctane sulfonatesulfonate (PFOS) and its perfluoro-analogues (PFAs) are of great concern due to their bioaccumulative nature, their persistence. 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. This resistance contributes to the extraordinary stability of PFOS and as well as PFAs. These compounds have been widely used over the last 60 years in industrial, commercial household applications and civilian production because of their unique physiochemical characteristics such as chemical (strong lipophobic and hydrophobic), thermal stability as well as surface active properties which account for their ability to make materials stain, oil, water resistant and defogger of car window and also incombustible treatment. The widespread applications, environmental persistence of PFAs have resulted in global occurrence of these substances in air, water, sediments and sludge with great concern. The height concentration in ground water was 29μM that is 14600 μg/l and that in such sewage sludge was 53.83 μg/g. Bioaccumulation of them in various wildlife species was reported. The highest PFOS concentration reported in a fish blood from Lake Biwa in Japan was 1.6 μM. An increasing number of studies report high levels of PFOS in human samples such as blood, tissues, and breast milk. A maximal level of PFOS (3.49 μg/mL=7μM) was found in the serum of retired worker. These results led to the increasing concerns about the potential detrimental effects of PFOS on human and wild animal's health.

An objective of the present study was to compare the vasocontractile effects of PFOS on isolated Wistar rats arterial ring preparations to clarify the toxicity of PFOS on tissue level.

MATERIALS AND METHODS

After being purchased ten of wistar strain rats at 8 weeks of age from a commercial supplier, they habituated for 2 weeks in the animal room before starting the experiment. PFOS and other chemicals of analytical reagent grade were purchased from a commercial sources.

The changes of tension of isolated arterial rings preparations was measured by force-displacement transducer. For rats arterioles ring preparation, rats were euthanized by carbon dioxide then thoracic aorta (TA), common carotid artery (CA), femoral artery (FA), pulmonary artery (PA), renal artery (RA) and supra-mesenteric artery (SMA) were excised rapidly, the adhering fat and connective tissues trimmed off, and cut carefully into ring preparation 3-4 mm in width. Cumulative concentration response curves for PFOS (1-100μM) were obtained in arterial ring preparations through using Krebs-Henseleit solution. The resting tensions were 0.4g for RA, 0.6g for SMA and FA, 0.8g for CA, 1.0g for PA and 1.2 g for TA, respectively. The ring preparation was connected to the lever of the transducer 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℃ 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 mM noradrenaline (NA) and washed to basic tension. Then, cumulative concentration response curves for PFOS (1-100 mM) 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, 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 stainless wire.

All experiments in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University and they were handled according to our institutional guidelines.

RESULT AND DISCUSSION

In our study, vasocontractile effects of PFOS on isolated Wistar strain male rat arterial rings were compared. The maximum contraction for PFOS on CA was larger than that of NA and it was most sensitive region and moreover 10μM of PFOS showed significant contraction. The contraction for PFOS was developed slowly and the tension kept stably. After washing, the tension

returned to the basic level

Regional difference on vasoconstriction effects of PFOS was also compared, 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 M of PFOS.

Concerning structure-activity relationship, PFOS was the most potent compared with PFOA, OS or OA in CA preparation. PFOS was more potent compared with PFOA in TA preparation. No significant effect was observed with the remove of endothelium on PFOS-induced contraction in CA preparation.

Concerning toxic effects of PFOS, PFOS was applied 2.0 mg/kg/day for 20 days during gestation and found mitochondrial injury in heart of dame, but not by 0.6 mg/kg/day in previous paper. They suggested the possibility of the developmental toxicity, but it seems to be very high dose compared with natural accumulation of PFOS. Other hand hepatic toxicity of PFOS wasreported, but non-observed-adverse effect level of PFOS was 1.25 mg/kg/day for 28 days. In vitro study the change of binding ratio of vitamin B_2 to serum albumin by PFOS at mM order concentration. Whereas our findings effective concentration (10μM) as relative with natural accumulations of PFOS showed significant vasoconstrictions. Recently the effects of 0.2μM of PFOS on Nanog mRNA and protein in mouse embryonic stem cell culture were reported. Embryonic damages may observe in μM or 0.1 μM order.

Concerning the occurrence of PFOS found in environment such as marine mammal's mink liver was 0.0595μg/g and polar bear was 4μg/g. The height PFOS concentration reported in a fish blood from Lake Biwa in Japan was 1.6 μM (834μg/l) and that in a fish liver in Okinawa was 7.9μg/g. A maximal level of PFOS (3.49 μg/mL~7 μM) was found in the serum of retired worker. The concentration of significant contraction by PFOS in CA of rats observd in the present study was nearly equivalent to the maximum contamination reported in human and wild animals.

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

In conclusion, present results indicated the possible toxicity of PFOS as an environmental contaminant and further studies on pollution of PFAs in the environment and toxic effects of them are necessary.