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Organophosphate agents induce plasma hypertriglyceridemia in mouse *via* single or dual inhibition of the endocannabinoid hydrolyzing enzyme(s)

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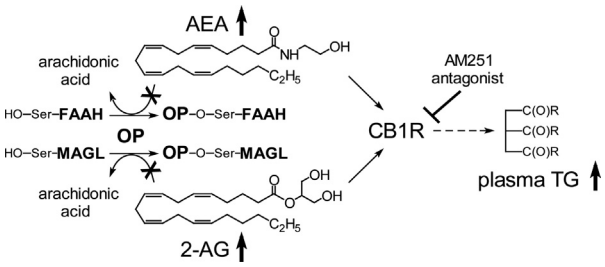
HIGHLIGHTS

- Organophosphate insecticide fenitrothion induces mouse plasma hypertriglyceridemia.
- The triglyceride elevation is prevented by the cannabinoid receptor antagonist AM251.
- Fenitrothion exposure leads to selective inhibition of the liver fatty acid amide hydrolase.

GRAPHICAL ABSTRACT



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ABSTRACT

Diverse serine hydrolases including endocannabinoid metabolizing enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) have been suggested as secondary targets for organophosphate (OP) agents to exert adverse toxic effects such as lipid homeostasis disruption. The goal of this investigation is to verify that a major OP insecticide fenitrothion (FNT) induces plasma hypertriglyceridemia through the inhibition of FAAH and/or MAGL in comparison with that elicited by isopropyl dodecylfluorophosphonate (IDFP), a potent FAAH/MAGL inhibitor. Fasted mice were treated intraperitoneally with FNT or IDFP and were subsequently sacrificed for evaluations of plasma triglyceride (TG) levels and liver FAAH/MAGL activities. Plasma TG levels were significantly enhanced by the FNT or IDFP treatment (1.7- or 4.8-fold, respectively) compared with that of vehicle control. The IDFP exposure reduced the liver FAAH and MAGL activities, whereas the FNT exposure led to the preferential FAAH inhibition. The brain acetylcholinesterase was almost unaffected by the FNT or IDFP treatment, thus leading to no neurotoxic sign. Intriguingly, the TG elevations were averted by concomitant administration with the cannabinoid receptor antagonist AM251. The present findings suggest that OP agents induce plasma hypertriglyceridemia in mouse through single or dual inhibition of FAAH or/and MAGL, apparently leading to overstimulation of cannabinoid signal regulating energy metabolism.

1. Introduction

Organophosphate (OP) agents have been the major insecticides for many decades and are indispensable tools in protecting crops, people, and animals from pest insect attack and disease transmission. OP compounds are nerve poison primarily acting at the

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acetylcholinesterase (AChE) as an inhibitor phosphorylating the serine hydroxyl residue of the catalytic triad (Casida, 2009; Casida and Durkin, 2013). Recent attention in OP toxicology has also been paid to diverse OP-sensitive serine hydrolase secondary targets including fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) (Casida and Quistad, 2004, 2005). FAAH and MAGL are hydrolyzing enzymes of endogenous cannabinoid agonists, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), respectively, which activate the cannabinoid type-1 receptor (CB1R). Modulation or overstimulation of the endocannabinoid signaling system may lead to unfavorable physiological effects: *i.e.*, neurotoxicity (Quistad et al., 2001, 2002); hyperactivity (Carr et al., 2011); spermatoxicity (Lewis and Maccarrone, 2009; Lewis et al., 2012; Noro et al., 2013; Suzuki et al., 2013); energy balance modulation (Cota et al., 2003; Gamage and Lichtman, 2012); and hypoalgesia, catalepsy, or hypomotility (Long et al., 2009; Nomura et al., 2008a; Quistad et al., 2006).

A specific concern is given in OP-induced lipid homeostasis disruption. The endocannabinoid system plays a pivotal role in energy balance, lipid homeostasis and food intakes (Bluher et al., 2006; Colombo et al., 1998; Cota et al., 2003; Engeli et al., 2005; Jbilo et al., 2005; Osei-Hyiaman et al., 2005). Actually, an OP agent, isopropyl dodecylfluorophosphonate (IDFP) (Fig. 1), enhances plasma triglyceride (TG) levels through inhibition of MAGL activities in the mouse liver, muscle, and adipose tissues (Ruby et al., 2008). However, the triggering mechanism underlying the OP-induced TG elevation has not been entirely defined. Therefore, the first aim of the present investigation is to verify that a major OP insecticide fenitrothion (FNT) (Fig. 1) also induces plasma lipid disturbances in mice. The second goal is to clarify the initial event(s) for the OP-induced TG elevation in comparison between actions of FNT and IDFP. Consequently, this study suggests that the OP-elicited plasma hypertriglyceridemia appears to be relevant to single or dual inhibition of FAAH or/and MAGL perhaps leading to overstimulation of CB1R-mediated signal regulating energy metabolism.

2. Materials and methods

2.1. Chemicals

Sources of the chemicals utilized in the present study are listed as follows: FNT and FNT oxon (Fig. 1) from Wako Pure Chemicals (Osaka, Japan); AM251 from Cayman Chemical (Ann Arbor, MI); radiolabeled substrates [^{14}C]AEA and [^{14}C]mono-oleoylglycerol from American Radiolabeled Chemicals (St. Louis, MO); AEA, and AEA-d_4 from Cayman Chemical and Abcam (Cambridge, MA), respectively; 2-arachidonoyl glycerol (2-AG) and 1-*O*-dodecyl-rac-glycerol (OG) from Cayman Chemical and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. IDFP was synthesized according to previous report of Segall et al. (2003a,b). For TG and total cholesterol (T-Chol) assays, enzyme reagent kits, triglyceride E-test and cholesterol E-test, were purchased from Wako Pure Chemicals.

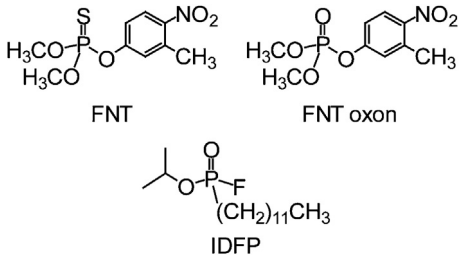

Fig. 1. Chemical structures of OP agents considered in the present investigation. A major OP insecticide FNT is transformed by cytochrome P450 to FNT oxon, therefore enabling to phosphorylate the serine hydroxyl residue at the catalytic triad of diverse serine hydrolases. IDFP is a potent FAAH or MAGL inhibitor (Nomura et al., 2008a; Quistad et al., 2006).

2.2. Animal studies

Throughout the animal experiments, the study was carried out in accordance with the Guide to Animal Experimentation of Nagoya City University (approval no. H23M-17). Male ICR mice aged 9 weeks were purchased from Japan SLC (Hamamatsu, Japan). The animals were housed in cages in the animal room under controlled environmental conditions: temperature 23–25 °C; relative humidity 57–60%; and a 12-h light–dark cycle (lighting: 9:00–21:00). Food and water were provided *ad libitum*.

Following 1 week of acclimation, the mice were fasted for 4 h and intraperitoneally administered vehicle (dimethyl sulfoxide), FNT (50 mg/kg), IDFP (20 mg/kg), or AM251 (10 mg/kg). In addition, AM251 (10 mg/kg) was simultaneously treated with FNT or IDFP. At the 3 h post-treatment, the fasted animals were sacrificed by decapitation. Blood was collected into heparinized tubes and then centrifuged to separate plasma. The resultant plasma and harvested liver and brain samples were stored at −80 °C until analyzed.

2.3. Plasma lipids

The plasma TG and T-Chol levels were assayed spectrophotometrically based on a glycerol-3-phosphate oxidase- or cholesterol oxidase-peroxidase enzymatic reaction using *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt (Allain et al., 1974; Spayd et al., 1978).

2.4. Enzyme assays

FAAH or MAGL activity in mice liver was assayed by hydrolysis of the corresponding substrate [^{14}C]AEA or [^{14}C]mono-oleoylglycerol, respectively (55 mCi/mmol for both substrates), according to the previous methods of Quistad et al. (2001, 2006). In brief, 100 mg mouse liver was homogenized in 50 mM Tris–HCl buffer (pH 8.0). The homogenate was centrifuged at 1000 × *g* and 4 °C for 10 min and the supernatant was then at 20,000 × *g* for 20 min. The 20,000 × *g* pellet was finally reconstituted in the Tris buffer. Then, the aliquot was incubated with 1 μM [^{14}C]AEA or [^{14}C]mono-oleoylglycerol for 30 min at 37 °C, the enzymatic reaction was terminated by addition of organic solvent (chloroform:methanol:hexane, 1.25:1.4:1.0) and 200 mM K_2CO_3. Subsequently, the radioactivity in the aqueous upper phase, as the amount of the [^{14}C]arachidonic acid or [^{14}C]oleic acid produced from the enzymatic reaction, was determined by liquid scintillation counter. *In vitro* potency of FNT oxon as an inhibitor of the FAAH or MAGL in the mouse liver preparation was also evaluated by the above procedure. Half maximal inhibitory concentration (IC_{50}) values were calculated by iterative nonlinear least-squares regression using Sigmaplot software ver. 8.0 (SPSS, Chicago, IL). Relative to brain AChE assay, the mouse brain was homogenized in 0.1 M Na_2HPO_4–HCl (pH 8.0), and then the AChE activity was measured spectrophotometrically with acetylthiocholine as the substrate and dithionitrobenzoic acid as the chromogen (Quistad et al., 2006).

2.5. LC–MS

Analysis of liver AEA and 2-AG levels was performed on a LC–MS/MS-8030 system (SHIMADZU, Kyoto, Japan) composed of a solvent delivery device (LC30AD), an autosampler (SIL-30AC), a system controller (CBM-20A), and a column thermostat (CTO-20A) based on the method reported by Zoerner et al. (2012) with a slight