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Metabolic products and pathways of fluorotelomer alcohols in isolated rat hepatocytes

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Abstract

Fluorotelomer alcohols (FTOHs; $CF_3(CF_2)_xC_2H_4OH$; where $x = 3, 5, 7, 9$) are a novel class of polyfluorinated contaminants, recently detected in the North American atmosphere, that are possible precursors to the series of perfluoroalkyl carboxylates (PFCAs) in human blood. An in vivo rat study validated earlier independent work that poly- and per-fluoroalkyl carboxylates were metabolites of FTOHs, but our detection of several novel metabolites prompted us to examine their pathways in greater detail using isolated rat hepatocytes. Using 8:2 FTOH (i.e. where $x = 7$) as a model compound, the metabolic products formed by isolated rat hepatocytes were identified, and three synthesized intermediates were incubated separately to elucidate the metabolic pathways. For 8:2 FTOH, a major fate was direct conjugation to form the *O*-glucuronide and *O*-sulfate. Using 2,4-dinitrophenylhydrazine (DNPH) trapping, the immediate oxidation product of 8:2 FTOH was identified as 8:2 fluorotelomer aldehyde (8:2 FTAL; $CF_3(CF_2)_7CH_2C(H)O$). 8:2 FTAL was transient and eliminated HF non-enzymatically to yield 8:2 fluorotelomer α,β-unsaturated aldehyde (8:2 FTUAL; $CF_3(CF_2)_6CF{=}CHC(H)O$) which was also short-lived and reacted GSH and perhaps other endogenous nucleophiles. Four polyfluorinated acid intermediates were also detected, including 8:2 fluorotelomer carboxylate (8:2 FTCA; $CF_3(CF_2)_7CH_2C(O)O^-$), 8:2 fluorotelomer α,β-unsaturated carboxylate (8:2 FTUCA; $CF_3(CF_2)_6CFCHC(O)O^-$), tetrahydroperfluorodecanoate ($CF_3(CF_2)_6(CH_2)_2CO_2^-$), and dihydroperfluorodecenoate ($CF_3(CF_2)_6CH{=}CHCO_2^-$). The pathways leading to 8:2 FTCA and FTUCA involve oxidation of 8:2 FTAL, however, the pathways leading to the latter two polyfluorinated acids remain inconclusive. The fate of the unsaturated metabolites, 8:2 FTUAL and FTUCA, included conjugation with GSH and dehydrofluorination to yield α,β-unsaturated GSH conjugates, and GS-8:2 FTUAL which was subsequently reduced to the corresponding alcohol. Perfluorooctanoate (PFOA) and minor amounts of perfluorononanoate (PFNA) were confirmed as metabolites of 8:2 FTOH, and the respective roles of β- and α-oxidation mechanisms are discussed. The analogous acids, aldehydes, and

Abbreviations: DHPFCA, dihydroperfluoroalkyl carboxylate; DNPH, 2,4-dinitrophenylhydrazine; FTAL, fluorotelomer aldehyde; FTCA, fluorotelomer carboxylate; FTOH, fluorotelomer alcohol; FTUAL, fluorotelomer α,β-unsaturated aldehyde; FTUCA, fluorotelomer α,β-unsaturated carboxylate; HNA, 4-hydroxynonenoic acid; HNE, 4-hydroxynonenal; HPLC/MS/MS, high pressure liquid chromatography tandem mass spectrometry; PFCA, perfluoroalkyl carboxylate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; THPFCA, tetrahydroperfluoroalkyl carboxylate

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conjugated metabolites of 4:2, 6:2, and 10:2 FTOH (i.e. where $x = 3$, 5, and 9, respectively) were also detected, and metabolite profiles among FTOHs generally differed only in the length of their perfluoroalkyl chains. Preincubation with aminobenzotriazole, but not pyrazole, inhibited the formation of metabolites from all FTOHs, suggesting that their oxidation was catalyzed by P450, not alcohol dehydrogenase.

Keywords: Metabolism; Hepatocytes; Reactive intermediates; Fluorotelomer alcohol; Perfluorinated acids; Glutathione conjugates

1. Introduction

The discovery that blood of the general human population contained organic fluorine compounds was first established in 1968 [1]. At that time, it was hypothesized that the unknown contaminants were similar in structure to perfluorooctanoic acid (PFOA) [2], however, it was not until 2001 that unambiguous identification and quantification of PFOA was reported in human blood by high pressure liquid chromatography tandem mass spectrometry (HPLC/MS/MS) [3]. Prompted by the detection of longer chained perfluoroalkyl carboxylates (PFCAs) in wildlife samples [4,5], Kuklenyik et al. [6] recently demonstrated that the blood of American adults is also contaminated with a homologous series of PFCAs ($CF_3(CF_2)_yCOO^-$, where $y = 7$–10), including perfluorononanoate (PFNA, $y = 7$), perfluorodecanoate ($y = 8$), perfluoroundecanoate ($y = 9$), and perfluorododecanoate ($y = 10$). Surprisingly, the exposure sources for all of these substances are not understood.

The US Environmental Protection Agency has appealed for data regarding the sources of PFOA due to the risk of adverse developmental effects in human offspring [7]. Chronic human exposure to PFCAs is also of concern given the non-genotoxic tumorogenicity of PFOA in rats [8] and the inhibitory effect of PFOA and perfluorodecanoate on gap-junction intercellular communication [9]. The toxicological information pertaining to PFCAs is limited largely to PFOA and perfluorodecanoate, however, PFNA and perfluoroundecanoate produce effects that are similar to those elicited by PFOA and perfluorodecanoate [10,11]. The half-life of PFOA in human blood is estimated to exceed 4 years [12], and although the pharmacokinetics of longer PFCAs has not been examined in humans, longer perfluoroalkyl chains equate to longer elimination half-lives in experimental animals [13–15]. It can be generalized that all PFCAs resist catabolism and phase II conjugation, and are poorly excreted in humans.

To mitigate any future identifiable risks associated with PFCAs, it is necessary to understand their source(s) of exposure. Human PFCA exposure may result from two broad hypothetical scenarios: (i) direct exposure to PFCAs in commercial products, household dust, or ingestion of food and water containing PFCAs, or alternatively, (ii) via similar exposure routes to precursor molecule(s) that can be metabolized to PFCAs. The only documented direct use of long-chain PFCAs, other than PFOA, is as polymerization aids in fluoropolymer processing [16], but they are also fluoropolymer thermolysis products [17]. These sources may result in some human exposure to PFCAs but these are not examined here. Rather, based on the widespread detection of a series of fluorotelomer alcohols (FTOHs; $CF_3(CF_2)_xC_2H_4OH$; where $x = 3, 5, 7, 9$) in ambient air [18,19], we hypothesize that the later route of exposure is responsible, at least in part, for current human PFCA concentrations. For example, it is established that 8:2 FTOH (e.g. where $x = 7$) is metabolized to PFOA in rats [20], however, it is unknown to what extent other PFCAs are also formed from FTOHs. It is also not known if reactive intermediates are formed, and hence if there are any additional adverse health consequences to be expected upon FTOH exposure.

FTOHs belong to a class of telomerized fluorochemicals, having an estimated global production of 5×10^6 kg/year [21], that find use in a diverse range of commercial and industrial applications including paints, coatings, polymers, adhesives, waxes, polishes, electronics, and caulks [22]. Presumably as a result of their widespread use, 6:2, 8:2, and 10:2 FTOH (e.g. $x = 5$, 7, and 9) are now widespread in the North American atmosphere and human exposure can be expected. Although the magnitude of human exposure to FTOHs has not been assessed, their widespread distribution in ambient air warranted a comprehensive