Elevated Serum Oleic Acid Epoxide Concentration in Acetaminophen (Paracetamol) Poisoning

Dimitrios Tsikas

Institute of Toxicology, Core Unit Proteomics, Hannover Medical School, 30623 Hannover, Germany

Correspondence: tsikas.dimitros@mh-hannover.de (D.T.)

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ABSTRACT | cis-9,10-Epoxyeic acid (cis-EpOA) is produced from oleic acid both by hepatic cytochrome P450 (CYP) enzymes and by reactive oxygen/nitrogen species (RONS). We hypothesized that overdosed acetaminophen (paracetamol) elevates the formation of cis-EpOA by inducing oxidative stress. We measured the concentration of circulating cis-EpOA in six acetaminophen suicide subjects. Acetaminophen and cis-EpOA were measured by high-performance liquid chromatography (HPLC) and gas chromatography-tandem mass spectrometry (GC-MS/MS), respectively. In serum samples of the acetaminophen-suicided persons, cis-EpOA (nM) and acetaminophen (µM) concentrations were 47 and 132, 65 and 921, 275 and 270, 300 and 762, 319 and 166, and 3723 and 185, respectively. There was no correlation between cis-EpOA and acetaminophen concentration. In acetaminophen self-poisoned patients, serum cis-EpOA concentration is increased compared to non-medicated healthy subjects. Acetaminophen is a weak inhibitor of CYP enzymes (e.g., 30% inhibition of CYP3A4 at 1000 µM). Suicidal acetaminophen seems to induce the formation of RONS which oxidize esterified oleic acid to cis-EpOA which is then hydrolyzed by secretory hepatic phospholipase A₂ (PLA₂) to free cis-EpOA. Dihydroxy-stearic acids, the hydrolase products of cis-EpOA, are known to inhibit the activity of several clotting factors. We hypothesize that alterations of the coagulation cascade seen after acetaminophen administration/intoxication are in part due to cis-EpOA and dihydroxy-stearic acids. Being a stable lipophilic epoxide, cis-EpOA is likely to inhibit enzymes of the vitamin K cycle.

KEYWORDS | Acetaminophen; Cytochrome P450; Epoxidation; Oleic acid; Paracetamol

ABBREVIATIONS | cis-EpOA, cis-9,10-epoxyeic acid; CYP; cytochrome P450; GC-MS/MS, gas chromatography-tandem mass spectrometry; HPLC, high-performance liquid chromatography; NAC, N-acetylcysteine; NAPQI, N-acetyl-p-benzoquinoneimine; PFB, pentafluorobenzyl; PLA₂, phospholipase A₂; QC, quality control; RONS, reactive oxygen/nitrogen species

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1. INTRODUCTION

Oleic acid, cis-9-octadecenoic acid, is the most abundant monounsaturated fatty acid in human blood [1]. Epoxidation of the single C9/C10 double bond of oleic acid by cytochrome P450 (CYP) enzymes leads to the formation of cis-9,10-epoxy-octadecanoic acid (cis-EpOA) [1–3]. The physiological roles of oleic acid and cis-EpOA are poorly investigated. Oleic acid has been proposed to exert vasoprotective actions and to play a role in immune function [1]. cis-EpOA lacks carcinogenic potential [4]. Oleic acid and cis-EpOA are mainly metabolized by β-oxidation. cis-EpOA is physiologically present in human blood and urine [1]. The detection of racemic cis-EpOA in lipids of human leukocytes suggests a free radical-catalyzed epoxidation of esterified oleic acid in lipids.

The first accurately measured basal concentration of cis-EpOA in human plasma was achieved by a validated GC-MS/MS after HPLC separation [5]. In ethylenediaminetetraacetic acid (EDTA)-conditioned plasma of healthy humans, cis-EpOA occurs in its free, non-esterified form at concentrations within the range of 30 to 50 nM. In end-stage liver disease, cis-EpOA plasma concentrations are lower (i.e., 10 to 40 nM). The lowest cis-EpOA plasma concentrations were measured in patients suffering from liver cirrhosis [3], suggesting liver as the main cis-EpOA-synthesizing organ. Various CYP isoforms including CYP2C9 and CYP3A4 have been shown to epoxidize oleic acid to cis-EpOA in vitro [3].

Given the hepatotoxicity of acetaminophen at high doses, we hypothesized that circulating cis-EpOA concentrations would be altered in subjects poisoned by acetaminophen. In the present study, we report on cis-EpOA concentrations in the blood of six female subjects after suicide by acetaminophen.

2. MATERIALS AND METHODS

2.1. Subjects

Six females (age range: 12 to 18 years) with suspected acetaminophen poisoning were included in the study (Table 1). Blood samples were obtained approximately at 4 h (n = 3), 5 h (n = 1), and 28 h (n = 1) after acetaminophen ingestion. The time point of acetaminophen ingestion by one patient was unknown. Two patients had been under N-acetylcysteine (NAC) treatment at the time point of blood sampling. Serum acetaminophen concentration ranged between 132 and 921 µM as measured by HPLC with UV detection (236 nm). The Ethics Committee of the Hannover Medical School was consulted. The study was performed according to the Declaration of Helsinki and the recommendations of the Central Ethics Committee for Medicine of the German Medical Association regarding the further use of human body materials for medicinal research purposes.

2.2. Quantification of cis-EpOA

cis-EpOA was quantitated in 1 ml aliquots of biological samples by GC-MS/MS using cis-[9,10-2H2]-EpOA (at 50 nM) as the internal standard [5]. The procedure includes solvent extraction with ethyl acetate (2 ml), derivatization by pentafluorobenzyl (PFB) bromide, and HPLC isolation of the PFB ester derivatives of cis-EpOA and cis-[9,10-2H2]-EpOA. Quantification was performed by selected-reaction-monitoring the mass transition m/z 297 to m/z 171 for cis-EpOA and m/z 299 to m/z 172 for cis-2H2-EpOA. Potential interference of cis-EpOA measurement by high acetaminophen concentrations was investigated by adding acetaminophen to human plasma samples at concentrations up to 1000 µM and by analyzing cis-EpOA by GC-MS/MS [5]. cis-EpOA in unspiked human plasma samples and samples spiked with acetaminophen (range: 0–1000 µM) was measured at 53.8 ± 2.9 nM, (RSD: 5.4%), suggesting no interference of cis-EpOA measurement by acetaminophen [5]. In parallel, we measured in triplicate the cis-EpOA concentration in unspiked plasma and plasma samples spiked with 16 nM cis-EpOA serving as a quality control (QC) sample. In the QC plasma sample, the acetaminophen concentration was less than 0.7 µM. The cis-EpOA concentration was determined to be 63.2 ± 4.6 nM in the unspiked plasma sample.
sample and 80.6 ± 3.5 nM in the spiked plasma sample (recovery: 109 ± 10%).

3. RESULTS

The serum concentration of acetaminophen in the poisoned subjects was 132, 166, 185, 270, 766, and 921 µM (Table 1). These values are higher than the maximum serum concentration (C_{max}) values obtained after oral administration of therapeutically relevant single doses of 500 to 1000 mg acetaminophen [6]. There was no correlation between cis-EpOA and acetaminophen concentrations in the patients’ blood. The highest serum cis-EpOA concentration of 3723 nM is about 100 times the mean plasma cis-EpOA concentration measured in healthy subjects [3]. Except for two patients, circulating acetaminophen concentrations were higher than the highest acetaminophen plasma concentrations measured in four healthy subjects after ingestion of 3000 mg acetaminophen at once [7].

4. DISCUSSION

Acetaminophen (paracetamol) is one of the most commonly used and misused drugs in many countries. In the six young females of our study, we measured toxicologically relevant concentrations of acetaminophen in blood drawn 4, 5, or 28 h after drug ingestion, including one patient being treated with NAC. In four patients, the concentration of circulating cis-EpOA was several-fold higher compared to healthy non-medicated subjects [3, 5].

cis-EpOA may originate from non-enzymatic and CYP-catalyzed reactions (Figure 1) [1–3]. Acetaminophen (1000 µM) was found to inhibit CYP3A4 activity (by 30%), a major CYP isoform contributing to cis-EpOA formation [3]. The temporary almost 6-fold increase in cis-EpOA seen in two healthy subjects who ingested 3 g acetaminophen at once could be due to an acetaminophen-induced short-term release of secretory hepatic phospholipase A2 (sPLA2) [7]. This may also have occurred in the acetaminophen-suicided persons of the present study.

Acetaminophen’s hepatotoxicity is associated with activity alterations of many enzymes. Diminished β-oxidation and decreased activity of microsomal and cytosolic epoxide hydrolases and glutathione S-transferases may lead to the accumulation of cis-EpOA [1]. The biological activities of cis-EpOA, 9,10-dihydroxy-stearic acid, and 9-hydroxy-10-glutathionyl-stearic acid are largely unknown. Acetaminophen poisoning was found to alter the activity of clotting factors and to increase the international normalized ratio (INR) [8]. N-Acetyl-p-benzoquinoneimine (NAPQI), the toxic metabolite of acetaminophen, was found to inhibit enzymes of the vitamin K cycle [9]. These observations raise safety issues in patients treated with vitamin K antagonists [10]. In rats, dietary 9,10-dihydroxystearic acid was found to induce a vitamin K-like deficiency syndrome which was reversed by 2-methyl-2,4-naphthoquinone (vitamin K_3) [11]. The similar effects caused by acetaminophen poisoning and 9,10-dihydroxy-stearic acid raise the question whether cis-EpOA itself or 9,10-dihydroxy-stearic acid also inhibits vitamin K cycle enzymes (Figure 1) and attenuates the activity of specific clotting factors [9, 10]. This remains to be elucidated in forthcoming studies. During vitamin K dependent γ-carboxylase catalysis, O_2 is transferred [12]. Such a mechanism would open the possibility of cis-EpOA formation.

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Time of APAP Analysis after Ingestion (h)</th>
<th>NAC Treatment</th>
<th>APAP (µM)</th>
<th>cis-EpOA (nM)</th>
</tr>
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<td>47</td>
</tr>
<tr>
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</tr>
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</tr>
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<td>No</td>
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<td>300</td>
</tr>
</tbody>
</table>
from oleic acid in liver microsomes independent of CYP enzymes.

Acetaminophen causes major bleeding events at therapeutically relevant doses in patients with recent ischemic stroke [13]. Possible mechanisms could involve the participation of acetaminophen metabolites such as NAPQI [10] or cis-EpOA, rather than thromboxane A₂ inhibition in platelets by acetaminophen [7].

5. CONCLUSION

In acetaminophen self-poisoned young subjects, serum cis-EpOA concentration is several fold higher compared to non-medicated healthy subjects. Suicidal acetaminophen may induce the formation of RONS that oxidize esterified oleic acid to cis-EpOA which is then hydrolyzed to free cis-EpOA. Reported acetaminophen-induced alterations of the coagulation cascade may be in part due to cis-EpOA and its hydrolysis product 9,10-dihydroxystearic acid. We hypothesize that cis-EpOA is an inhibitor of enzymes of the vitamin K cycle, but this remains to be demonstrated.

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The author declares no conflicts of interest.
REFERENCES


