Fatty acid ethyl esters: recent observations

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Summary  Fatty acid ethyl esters (FAEE), esterification products of fatty acids and ethanol, have been shown to be mediators of ethanol-induced cell injury and their presence in the blood and tissues is a marker of ethanol intake. Recently, it has been shown that FAEE are produced within seconds of infusion of ethanol into the heart, when using a protocol similar to that used for myocardial ablation. This raises the possibility that the mechanism for the death of myocytes in cardiac ablation involves the generation of toxic FAEE. It has also been recently demonstrated that chronic alcoholics have a high concentration of a specific FAEE species—ethyl oleate. The use of the serum ethyl oleate concentration may be helpful in differentiating binge drinkers from chronic alcoholics. © 2002 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Fatty acid ethyl esters (FAEE) are esterification products of fatty acids and ethanol.1,2 It has been demonstrated in an autopsy study that the organs most frequently damaged by ethanol abuse, namely the pancreas, liver, heart, and brain, are the organs with the highest concentrations of FAEE after ethanol intake, and these organs were shown to have the highest concentrations of the enzymes responsible for FAEE synthesis.3

FAEE have been demonstrated to be toxic both in vitro4 and in vivo.5 In the in vitro studies, HepG2 cells exposed to FAEE in the core of low density lipoprotein (LDL) particles incorporated the FAEE containing LDL, and the FAEE impaired the proliferation of HepG2 cells and decreased the synthesis of cellular protein.4 In vivo, FAEE in the core of LDL lipoprotein particles at an 11 μM FAEE concentration (attainable by ingestion with 5–10 ethanolic drinks), caused an increase in trypsinogen activation peptide, a marker of pancreatic cell injury in rats.5 We have recently completed or are nearing completion of a variety of studies involving FAEE. The remainder of this report summarizes this most recent body of work (Table I).

RECENT BIOCHEMICAL OBSERVATIONS ABOUT FAEE

Patients who have outflow obstruction from the left ventricle due to excess myocardial tissue have a new therapeutic option to remove the tissue, which is much less invasive than open cardiac surgery. Catheter-directed myocardial ablation involves the infusion of 100% ethanol (pure ethanol) to create a localized myocardial infarction. The controlled and localized destruction of excess myocardial tissue removes the outflow obstruction from the left ventricle. We raised the question whether FAEE is a mediator of cardiac myocyte cell death in this process. Initial studies have been performed with pigs to determine if there is FAEE production by the isolated heart, and if the FAEE appear within a time frame rapid enough to account for the effect produced by the ethanol infusion. We found substantial production of FAEE within minutes when the pig heart was infused via the coronary arteries with ethanol. Blood return from the cardiac tissue (that had not been through the systemic circulation) was collected from the coronary sinus. At peak concentration, the blood ethanol levels from this collection site were on
the order of 500–600 mg/dl, while the values in the peripheral circulation were 35–45 mg/dl. The FAEE emerging from the coronary sinus peaked at approximately 1.6 μM, with a peak peripheral FAEE concentration of approximately 1.6 μM. The 12 μM FAEE concentration from coronary sinus blood was present in a sample diluted by blood already in the heart at the time of ethanol infusion. Thus, the FAEE concentration at the site where the ethanol directly interacts with the myocardium is much greater, but its actual concentration is difficult to precisely determine. An FAEE concentration of 11 μM has been shown to produce pancreatic acinar cell injury. Therefore, the much higher local FAEE concentration generated at the site where ethanol contacts myocardial tissue is likely to have significant toxicity.

Pathways of non-oxidative ethanol metabolism are more newly discovered than the oxidative pathways. We became interested in knowing if ethanol metabolism toward FAEE increased when the oxidative pathways are inhibited. We have demonstrated both in vitro and in vivo that inhibition of ethanol oxidation by inhibitors of alcohol dehydrogenase, inhibitors of cytochrome P450, and catalase—all of which can catalyze the oxidation of ethanol to acetaldehyde—results in an increase in ethanol flux toward FAEE, both in the liver and in pancreas. When these inhibitors were delivered to the living rat or to homogenized rat organs exposed to ethanol, they increased FAEE production. The relative toxicity of different FAEE species is unknown. We have previously reported that the fatty acid composition of FAEE found in human plasma after ethanol ingestion is predominantly ethyl palmitate (E16:0) and ethyl oleate (E18:1). This selectivity for certain species of FAEE in the blood, based upon the fatty acid present, raised the question of whether the liver and pancreas secrete certain species of FAEE into the bile and pancreatic secretions, respectively, while retaining other species within the cell. We recently found in an animal study that there is selectivity for certain FAEE species to be secreted into the blood and the bile by the liver, and selectivity for certain FAEE species to be secreted by the pancreas into the pancreatic duct. The biochemical basis for this selectivity remains unknown.

### RECENT OBSERVATIONS ON THE DIAGNOSTIC UTILITY OF FAEE IN THE ASSESSMENT OF ALCOHOL INTAKE

FAEE are transported in the blood in the core of lipoproteins and bound to albumin. FAEE in the core of lipoproteins rapidly exchange into phospholipid bilayers. We have previously reported that the FAEE align parallel to the fatty acid moieties of phospholipids in bilayers and are soluble up to 30 mol% percent of the fatty acids within the phospholipids. FAEE are primarily bound to albumin at low blood concentrations of FAEE, but as the FAEE concentration increases in the blood, the percentage of blood FAEE associated with lipoproteins becomes much greater. This is not surprising since lipoproteins can carry several thousand molecules, and an albumin molecule carries a few at most. We have recently demonstrated that increasing the ratio of free fatty acid to ethyl oleate from 0.1 to 10 is associated with a displacement of ethyl-14C oleate bound to albumin (unpublished observation, Best C. A., Szczepiorkowski Z. M., Laposata M., May, 2001). Thus, fatty acids are preferred over FAEE in the binding to albumin.

We have learned that albumin, VLDL, LDL, and HDL all stimulate the release of FAEE from cells induced to synthesize FAEE by exposure to ethanol. We have shown that adding albumin, LDL, or HDL to the medium of HepG2 cells increases the extraction of FAEE from the cells in a time- and dose-dependent manner. We also found that an increase in these FAEE carrier concentrations

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Table 1 Recent observation in studies on fatty acid ethyl esters

<table>
<thead>
<tr>
<th>Observation</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Biochemical</td>
<td>Yoeger (2002)^6</td>
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<td>FAEE are generated rapidly in high concentration by the isolated pig heart in vivo</td>
<td>Soderberg et al. (1999)^16</td>
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<tr>
<td>Inhibition of ethanol oxidation by inhibitors of alcohol dehydrogenase, cytochrome P450, and catalase, with increase ethanol flux towards FAEE in the liver and pancreas</td>
<td>Werner (2001)^7</td>
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<td>There is selectivity in the FAEE species secreted by liver and the pancreas</td>
<td>Laposata (2000)^9</td>
</tr>
<tr>
<td>Diagnostic</td>
<td>Refaai et al. (2001)^10</td>
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<td>FAEE are higher in men than women for an equivalent weight-based ethanol intake</td>
<td>Salem et al. (2001)^18</td>
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<tr>
<td>Ethyl oleate is higher in chronic alcoholics than in episodic drinkers after ethanol intake</td>
<td>Salemi et al. (2001)^18</td>
</tr>
<tr>
<td>FAEE concentrations in liver and adipose tissues can be used as post-mortem markers for ethanol intake</td>
<td>Zybko et al. (2001)^21</td>
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<td>Methodology</td>
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<td>Plasma samples must be collected in anticoagulants other than EDTA; serum samples are acceptable; plasma or serum samples should be analyzed or frozen within 4 h of collection</td>
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<td>Improved sensitivity for FAEE detection can be achieved by reducing the sample size relative to the volume of extracting solvents</td>
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elevates FAEE production by HepG2 cells and that the cells retain a fixed amount of FAEE, independent of acceptor concentration and time of incubation.14

FAEE can be used as long-term markers of ethanol intake, after ethanol is undetectable in the circulation. We have previously demonstrated in a clinical study in which individuals were given approximately six alcoholic drinks to consume over a 90 min period, with frequent blood collections through a peripheral vein catheter, that the increase and decrease of blood alcohol and FAEE concentrations essentially overlap. However, the one important difference was that the FAEE in the serum were detectable for up to 24 h while the ethanol was not.15 Despite the fact that ethanol concentrations in women have long been known to be higher than in men for a given weight-based ethanol intake, we have recently reported that the FAEE peak concentration is approximately 2-fold higher in men than in women given equal weight-based amounts of ethanol.16

In some of our earlier work, we made the observation that chronic alcoholics appear to have a higher concentration of ethyl oleate than episodic (binge) drinkers, following ethanol intake. Therefore, we performed a study to determine if the fatty acid in FAEE can be used to differentiate between chronic alcoholics and episodic heavy drinkers (binge drinkers).17 The clinical management of these two groups of patients is significantly different, as episodic heavy drinkers have greater mortality and morbidity acutely from very high concentrations of ethanol than chronic alcoholics. We showed in this investigation that the ethyl oleate concentrations of chronic alcoholics and episodic heavy drinkers are significantly different at or near peak ethanol concentrations. The ethyl oleate levels were much higher in chronic alcoholics than in the episodic heavy drinkers (P > 0.0001). We also demonstrated that the concentration of ethyl oleate in chronic alcoholics and binge drinkers approximately 24 h after ethanol consumption was discontinued was significantly different, with the chronic alcoholics having a much higher ethyl oleate concentration.

Because blood is not always available for post-mortem assessment of ethanol intake before death, we have begun to assess the diagnostic value of FAEE in solid organs and tissues as markers of ethanol intake. In one study, rats given an interperitoneal injection of ethanol, and then allowed to survive for several hours until sacrifice, provided samples of liver and retroperitoneal adipose.18 Both the liver and the adipose showed the presence of FAEE, with the control animals that received no ethanol having absent or trace levels of FAEE. The presence of FAEE in solid organs in rats after exposure of ethanol led us to perform a human study in which 31 cases from the medical examiner’s office and our hospital department of pathology were evaluated, with post-mortem intervals ranging from 5 to 29 h (mean of 16 h).19 In all of these cases, blood ethanol concentrations were performed at autopsy to determine if the presence of FAEE predicted ethanol intake, which was objectively documented in these cases by blood ethanol measurement. Liver and adipose tissue FAEE were extracted and quantitated. Chronic alcoholics with a negative blood ethanol at autopsy and social drinkers with a negative blood ethanol at autopsy showed low liver FAEE values that were completely differentiated (with the exception of the one outlier) from those who had a detectable blood ethanol at autopsy and higher FAEE levels. Adipose tissue FAEE levels showed overlap between the chronic alcoholic group and the individuals with detectable blood ethanol at autopsy because the FAEE are stored in adipose tissue. The social drinkers had much lower adipose FAEE levels than the other two groups. The FAEE concentration in human liver and adipose tissue has potential for use in clinical and legal decision making with regard to the determination of pre-mortem ethanol intake.

RECENT METHODOLOGIC ADVANCES IN THE ISOLATION AND QUANTITATION OF FAEE

If blood FAEE are to be used as a marker of ethanol intake, the stability of FAEE in the sample is an important issue. In a recent study with human blood samples, we investigated the effects of collection tube, storage time, and storage temperature on FAEE concentrations in blood. The results indicated a need for removal of the plasma or serum from the cells within 4 h at room temperature, and freezing of plasma or serum if more than 4 h is required before analysis of the specimen. We found that samples should not be collected for FAEE analysis in EDTA-containing (purple top) vacuum tubes, as it lowers the FAEE concentration by an unknown mechanism.20

We have improved the sensitivity of our FAEE quantification assay by reducing the sample volume size from 1.0 ml of sample to 0.5 ml and increasing the relative amount of solvent used for the first step in the extraction.21

Thus, many new biochemical, diagnostic, and methodologic advances related to FAEE have appeared recently which continue to enhance our understanding of this interesting ethanol metabolite.

REFERENCES


