THE USE OF PHOSPHATIDYLETHANOL (PETH) AS A DIRECT ALCOHOL BIOMARKER

AN ANNOTATED BIBLIOGRAPHY

The Use of Phosphatidylethanol as a Direct Alcohol Biomarker: An Annotated Bibliography

Introduction

Phosphatidylethanol (PEth), a group of abnormal phospholipids, are formed in the presence of ethanol, phosphatidylcholine, and the enzyme phospholipase D. PEth is considered a direct alcohol biomarker because of the incorporation of the original ethanol moiety in the final product. Structurally, PEth consists of a phosphoethanol head connected to 2 fatty acid moieties which determine the specific isomer. The palmitoyl/oleoyl species (POPE) was shown as the most prevalent isomer of PEth (40% of total PEth). Once formed, PEth incorporates into the phospholipid membranes of blood and tissue cells and decomposes with a half-life of 4-5 days, which allows for a wide window of detection.

Phosphatidylethanol Synthesis
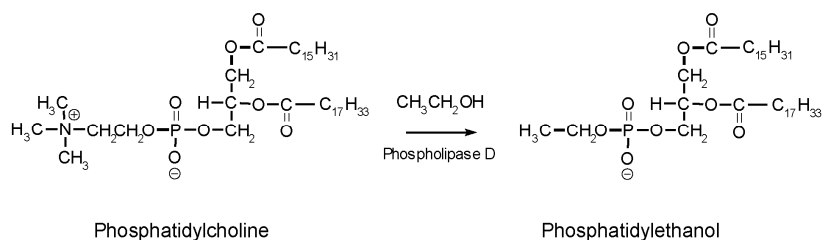
PEth has been gaining popularity as a direct ethanol biomarker to identify alcohol consumption. A number of organizations are currently using PEth including numerous healthcare professional programs, family courts, drug courts, DWI/OWI court programs, and residential treatment centers. Now that PEth is routinely available, numerous academic research institutions are using PEth to remove or re-categorize alcohol drinkers in their research cohorts. The National Institute on Alcoholism and Alcohol Abuse (NIAAA) has been an active participant in the development of PEth and has funded a significant number of studies and pilot programs.

Phosphatidylethanol Structure and LCMSMS Product Ion Fragments
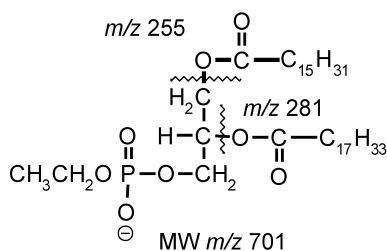
The purpose of this annotated bibliography is to compile into a single document the scientific journal articles that we feel are important in the history and development of PEth as a commercial analytical assay. There are many more articles in the scientific literature and we could not include them all but these are the papers that in our opinion are most important. Each article is headed by the official citation of the article so that you may locate this reference if you desire to read more in-depth. Each citation is followed by the published abstract which is a brief synopsis of the journal article. Lastly, we include a brief comment on the implication of the study or why we felt that this journal article was important.

ANNOTATED BIBLIOGRAPHY: PHOSPHATIDYLETHANOL (PEth)

1983 (1)

An Abnormal Phospholipid in Rat Organs After Ethanol Treatment

ABSTRACT

No abstract with this short communication.

Implications of this article

This article is a report on the appearance of an abnormal lipid in various rat organs following ethanol treatment, and the relation to the amount and duration of ethanol administration.

1987 (2)

Formation of Phosphatidylethanol in Rat Brain by Phospholipase D

ABSTRACT

The mechanism of phosphatidyl [^{14}C]ethanol formation was studied in rat brain microsomal fraction. Phospholipase D and base-exchange enzymes were assayed with [^{14}C]ethanol as substrate. Phospholipase D was found to catalyse the formation of phosphatidylethanol. The reaction was dependent on sodium-oleate as activating factor. Phosphatidylethanol formation by phospholipase D has previously only been reported to occur in plant tissues. Stimulation of base-exchange enzymes with calcium in the presence of [^{14}C]ethanol did not induce any formation of phosphatidylethanol. These findings indicate that phosphatidylethanol formation in ethanol intoxicated rats is catalysed by phospholipase D.

Implications of this study

This study investigated the mechanism of the formation of PEth via phospholipase D.

12-O-tetradecanoylphorbol-13-acetate Activates The Synthesis of Phosphatidylethanol in Animal Cells Exposed to Ethanol

ABSTRACT

Tumor-promoting phorbol esters acutely activate a pathway in lymphocytes leading to the synthesis and accumulation of phosphatidylethanol, using exogenous ethanol as a precursor. This product is a representative of a unique class of acidic glycerophospholipids in which the head group is a primary alcohol. The formation of this lipid, in response to different phorbol ester derivatives, correlates with their activity as tumor promoters and Inducers of growth changes in a variety of animal cells. Since phosphatidylethanol represents an unusual metabolite of ethanol, it is proposed that studies of its synthesis and biological functions may also provide new perspectives on the biology of alcohol addiction as well as the role of this biological pathway in tumor promotion.

Implications of this study

One of the first reports indicating that PEth is a direct alcohol biomarker.

Synthesis of Phosphatidylethanol - A Potential Marker for Adult Males at Risk For Alcoholism

Mueller, G., Fleming, M., Lemahieu, M., Lybrand, G., & Barry, K. (1988). *Proceedings of the National Academy of Sciences,* 85, 9778-9782.

ABSTRACT

Phosphatidylethanol, whose synthesis is catalyzed by a phospholipase D in a transphosphatidylation reaction, is a unique metabolite of ethanol. Phorbol 12-tetradecanoate 13-acetate, a tumor-promoting phorbol ester and stimulator of protein kinase C, activates this enzyme in peripheral blood lymphocytes. This system has been developed into an assay for measuring the potential of this pathway in human subjects. A pilot study of phosphatidylethanol synthesis in lymphocytes of adult males who have both an alcohol dependency and a family history of alcoholism has revealed that the average potential for phosphatidylethanol synthesis in this population is significantly elevated over that of control subjects.

Implications of this study

An early report of the synthesis of PEth by phospholipase D in the presence of ethanol.

Blood Phosphatidylethanol as a Marker of Alcohol Abuse: Levels in Alcoholic Males During Withdrawal

ABSTRACT

Phosphatidylethanol (PEth) is formed only in the presence of ethanol, via the action of phospholipase D. We studied PEth in blood as a possible marker of alcohol abuse in 15 male alcoholics admitted for detoxification. Blood was drawn on the first day after admission and up to 28 days thereafter. PEth in whole blood was 13.2 ± 2.2 pmol liter^{-1} (mean $\pm$ SE) at first sampling and remained detectable up to 14 days after admission. Blood ethanol was 0 on the morning after admission. The time courses of PEth disappearance varied among individuals. No PEth could be found in blood of control persons who had abstained from ethanol for 4 days. Levels of PEth and carbohydrate- deficient transferrin or y-glutamyltranspeptidase did not correlate. Its high specificity and prolonged detectability suggest PEth in blood as a marker of recent alcohol abuse.

Implications of this study

This study was the first report of the use of PEth to distinguish chronic alcoholics from teetotaler controls using thin layer chromatography.

Determination of Phosphatidylethanol in Blood From Alcoholic Males Using High-Performance Liquid Chromatography And Evaporative Light Scattering or Electrospray Mass Spectrometric Detection

ABSTRACT

The 'pathologic' phospholipid, phosphatidylethanol (PEth), formed only in the presence of ethanol, was determined in extracts of human blood using high-performance liquid chromatography with evaporative light scattering detection (ELSD) or electrospray (ES) mass spectrometry. Separation was performed using a diol column and a normal-phase binary gradient system. Decreasing concentrations of PEth (15 to 1 nmol/ml blood) could be detected by ELSD in three male alcoholics, up to 3 weeks after the beginning of an alcohol-free period. Using ES, levels down to 100 pmol/ml blood was detected. The molecular species of PEth were similar to those of phosphatidylcholine found in the same blood sample. The method provides a rapid quantitative and qualitative determination of PEth in blood. The limits of detection were 200 pmol (ø125 ng) using ELSD and 140 fmol (ø100 pg) using ES, total amounts injected.

Implications of this study

This was the first report of a validated HPLC-Evaporative Light Scattering Detector method and a HPLC-Mass Spectrometry (single quadrupole) method to detect PEth in the blood of known chronic alcoholics.

Phosphatidylethanol in Blood as a Marker of Ethanol Consumption in Healthy Volunteers: Comparison With Other Markers

ABSTRACT

Phosphatidylethanol is a "pathological" phospholipid, formed via the action of phospholipase D only in the presence of ethanol. The present study was made to elucidate how different levels and patterns of alcohol intake affect blood levels of phosphatidylethanol in comparison with other markers of abuse. We used a new HPLC-evaporative light-scattering detection technique for phosphatidylethanol quantitation. This method had a total coefficient of variation of < 20% at the detection limit of 0.2 nmol, equaling 0.8 μmol/liter of whole blood. Two groups were studied. (a) Five healthy volunteers were given 32 to 47 g of ethanol in a single dose, to give blood ethanol levels of ~25 mmol/liter after 30 to 60 min. Phosphatidylethanol, carbohydrate-deficient transferrin (CDT), and blood ethanol were measured before and after the intake. (b) Twelve student volunteers were studied during a 3 week period of prolonged alcohol consumption (total estimated intake: 1334 ± 488 g, mean ± SD) and phosphatidylethanol, serum-CDT, γ-glutamyltransferase, and blood ethanol were measured at the start of the period (day 1) and twice at the end of the period (days 18 and 21). In group (a), no phosphatidylethanol was detected at any time after ethanol dosage/take. In group (b), no blood phosphatidylethanol or blood ethanol could be demonstrated at the start, and serum-CDT was below the discrimination limit (1.3%) in all persons. No phosphatidylethanol was detected in those four persons with the lowest intake (742 ± 150 g). However, the remaining eight persons had detectable levels of phosphatidylethanol (1.0 to 2.1 μmol/liter), and these had a higher total intake (1630 ± 389 g). There was a statistically significant (p= 0.02) increase in serum CDT for 3 weeks. However, only 3 of 12 persons increased above the discrimination limit. The present results indicate that a substantial alcohol intake is needed to elevate blood phosphatidylethanol. In comparison with serum-CDT, blood phosphatidylethanol appears more sensitive.

Implications of this study

A comparison of PEth and CDT demonstrated that PEth outperformed CDT in sensitivity and specificity.

Normalization Rate And Cellular Localization of Phosphatidylethanol in Whole Blood From Chronic Alcoholics

Varga, A., Hansson, P., Johnson, G., & Alling, C. (2000). *Clinica Chimica Acta,* 299, 141-150.

ABSTRACT

Phosphatidylethanol (PEth) is an abnormal phospholipid which is formed in the presence of ethanol, via the action of phospholipase D (PLD). PEth in blood is a potential marker of alcohol abuse. The present study was made to determine the compartmentalization and the elimination rate of PEth in human whole blood. PEth was assayed by an improved HPLC technique, with evaporative light-scattering detection. Blood from six alcoholic males was separated into different blood cell fractions. The PEth concentration in whole blood was 2.5 ± 0.9 and 1.9 ± 1.1 mmol/ l in erythrocytes. Only one subject had detectable PEth in the mononuclear cells. Fifteen patients (13 men, two women) with chronic alcoholism, were followed as inpatients, after admission to an alcohol detoxification clinic. PEth, carbohydrate-deficient transferrin (CDT) and g-glutamyltransferase (GGT) were measured on days 1, 3, 5 and 7 after admission. Linear regression analysis of logarithmic PEth values in individuals, with measurable PEth at day 1, gave a good fit ($P < 0.001$) with the one-compartment elimination model. The half-life was calculated as 4.0 ± 0.7 days. A weak significance ($P < 0.05$) was observed in the correlation of PEth at day 1 and half-life values of the same subjects.

Implications of this study

This study demonstrated that PEth in the blood resides primarily in erythrocytes. The authors further calculated that the rate of disappearance had a half-live of a little over 4 days.

Analysis of Trimethylsilyl Derivatization Products of Phosphatidylethanol by Gas Chromatography-Mass Spectrometry

ABSTRACT

For the detection of rare phospholipid, phosphatidylethanol (PEt), GC-MS analysis method was adopted for the detection of derivatization products of PEt by N,O-bis (trimethylsilyl) trifluroacetamide (BSTFA). A re-structured molecule derived from PEt, ethyl bis (trimethylsilyl)-phosphate was found from search of Wiley database. This molecule can be used as a marker for PEt analysis. The molecular formula was $C_8H_{23}O_4PSi_2$ and weight of the formula was 270.09.

Implications of this study

This was the first and only published method using GCMS for the detection of PEth.

Formation of Phosphatidylethanol *in vitro* in Red Blood Cells From Healthy Volunteers And Chronic Alcoholics

ABSTRACT

Phosphatidylethanol (PEth) is an abnormal phospholipid, formed only in the presence of ethanol via a transphosphatidylation reaction of phospholipase D (PLD). PEth in blood is a promising new marker of alcohol abuse. Blood PEth is found almost exclusively in red cells. This study was performed to investigate a possible PEth formation in human red cells from alcoholics and healthy individuals, at physiologically relevant ethanol concentrations. Blood was drawn from six healthy volunteers (controls) and six chronic inpatient alcoholics. Hematological analyses were performed, and red blood cells were separated and incubated in plasma with ethanol to study PEth formation. Lipids were extracted and PEth analyzed with high pressure liquid chromatography and evaporative light-scattering detection. Incubation of red cells in 50 mM ethanol yielded detectable PEth after 12 hours. Formation of PEth was concentration dependent at 10 to 50 mM ethanol. *In vitro* formation of PEth was significantly higher (P < .001) in red cells from alcoholics (5.2 ± 1.1mol/l) compared to controls (2.4 ± 0.6 mol/l) (mean ± SD). A significant correlation (P < .01) was observed between initial mean corpuscular volume and accumulated PEth. This study demonstrates that PEth is formed in human red cells at physiologically relevant ethanol concentrations. Alcoholics accumulate about twice as much PEth than controls. The accumulation rate of PEth is slower in red cells compared to rates reported for other tissues.

Implications of this study

This study compared PEth results between alcoholics and teetotalers and determined that PEth is formed only in the presence of significant blood ethanol concentrations.

Advanced Lipid Extraction Method For The Determination of The Phospholipase D Activity

ABSTRACT

Phospholipase D is a ubiquitous enzyme that plays an important role in various lipid mediated cellular signaling pathways and produces rare phospholipids, phosphatidylethanol or phosphatidylbutanol, instead of phosphatidic acid with unique catalytic activity transphosphatidylation in the presence of primary alcohols. The reaction products, phosphatidylethanol or phosphatidylbutanol are used as markers of *in vitro* phospholipase D activity in many studies. For the sensitive detection of the phospholipase D products, we developed an advanced lipid extraction method that facilitates recovery of the compounds. With the new method, the activity change of phospholipase D by agonists could be detected more easily and the recovery rate was also increased. The increase of detected enzyme activity change was about double fold compared to the conventional lipid extraction method. This method provides selective force for the phospholipase D products in the extraction procedure.

Implications of this study

This article presented an improved method for the extraction of PEth from blood.

Phosphatidylethanol Formation and Degradation in Human And Rat Blood

ABSTRACT

Aims: To investigate the rate of formation and degradation of phosphatidylethanol (PEth) in rat blood as compared to human blood, as a model for a biological marker for ethanol exposure.

Methods: Rats were given 9% ethanol in liquid diet for 30 days. Control rats were pair fed with a control liquid diet. Blood and organs were analysed considering PEth formed *in vivo*. Blood from man, rat, pig and ferret as well as human HepG2 cells and rat C6 glioma cells were studied with respect to formation and degradation of PEth *in vitro*. PEth was analysed by high performance liquid chromatography (HPLC).

Results: Most rat organs accumulated considerable amounts of PEth whereas no PEth was found in the blood. After *in vitro* incubations of blood with ethanol, PEth was only formed by human blood, in contrast to the other species studied. HepG2 cells and C6 cells, like human blood, formed PEth *in vitro* but only the two cell lines had enzymatic degradation of PEth.

Conclusions: The rat is not suitable as a model for assaying PEth in blood as a consequence of ethanol intake. Human blood seems to be particular in its ability to synthesize PEth and to maintain a stable level of PEth due to the lack of degrading activity.

Implications of this study

This study demonstrated that PEth can be synthesized *in vitro* in human blood if the blood is exposed to ethanol. This study also demonstrated that PEth accumulated in the organs and tissues of various small mammals but PEth was not stable in rat blood. In contrast, once formed, PEth appeared to be very stable in human blood.

Phosphatidylethanol in Human Organs And Blood: A Study on Autopsy Material And Influences by Storage Conditions

ABSTRACT

Objective: Phosphatidylethanol (PEth) is an abnormal phospholipid that is formed and accumulated in mammalian cells that have been exposed to ethanol. PEth has been proposed as a marker of ethanol abuse. This study was conducted to investigate the concentration of PEth in blood and organs obtained during the autopsy of alcoholics. In addition, we performed experiments on rat tissues and human blood to evaluate the effect of various storage conditions on PEth concentrations.

Methods: Human tissues and blood from alcoholics and controls were obtained at autopsy and frozen at -20°C until extraction. Blood from healthy donors was incubated with ethanol for 24 hr and thereafter either extracted directly or stored at room temperature, stored at 4°C, frozen at -20°C, or frozen in liquid nitrogen and stored at -80°C before extraction. Rats were given intraperitoneal injections of ethanol and then killed, either while still intoxicated or when sober. Rat organs were homogenized and extracted directly, after a period of storage, and/or after freezing at -20°C. PEth concentration was analyzed using HPLC and verified by mass spectrometry.

Results: In all rat organs studied, PEth was formed during freezing at -20°C with ethanol present. PEth concentrations of 9 to 205 μmol/liter were observed in the blood obtained at autopsy. The highest value was found in the case with the highest blood alcohol concentration (114 mmol/liter) at the time of death. In the experiments on human blood stored with ethanol present, PEth concentrations were not affected after 72 hr at 4°C or after freezing in liquid nitrogen and storage at -80°C for up to 144 hr but were slightly elevated after 24 hr at room temperature and at -20°C. PEth was found in all organs obtained from the cadavers of alcoholics. Storage of organs at 4°C for 24 hr with ethanol present had no effect on the PEth concentration. The PEth concentration was unaffected when no ethanol was present at the time of freezing.

Conclusions: The rat experiments indicated that the very high PEth concentrations found in the organs of the alcoholics were probably largely formed while the organs were frozen at -20°C. Our data suggest that tissue material from bodies that were exposed to ethanol must be stored properly to obtain reliable results from subsequent analysis for PEth. Tissue should not be frozen at -20°C but instead stored refrigerated until extraction, preferably within hours of autopsy, or frozen in liquid nitrogen and stored at -80°C. Blood samples that contain ethanol can be stored refrigerated for up to 72 hr or frozen in liquid nitrogen and stored at -80°C without affecting PEth levels.

Implications of this study

The organs of rats synthesized measureable concentrations of PEth when harvested during a period of measureable blood alcohol concentration. The autopsied organs of alcohol related deaths contained measureable levels of PEth. The production of PEth *in vitro* is enhanced when frozen at -20°C because the water is solid and the ethanol remains a liquid, is concentrated and is available for synthesis. Blood stored at 4°C and -80°C minimized PEth *in vitro* production.

Methodological Modifications on Quantification of Phosphatidylethanol in Blood From Humans Abusing Alcohol, Using High-Performance Liquid Chromatography And Evaporative Light Scattering Detection

ABSTRACT

Background: Phosphatidylethanol (PEth) is an abnormal phospholipid formed slowly in cell membranes by a transphosphatidylation reaction from phosphatidylcholine in the presence of ethanol and catalyzed by the enzyme phospholipase D. PEth in blood is a promising new marker of ethanol abuse depending on the high specificity and sensitivity of this marker. None of the biological markers used in clinical routine at the present time are sensitive and specific enough for the diagnosis of alcohol abuse. The method for PEth analysis includes lipid extraction of whole blood, a one-hour HPLC separation of lipids and ELSD (evaporative light scattering) detection of PEth.

Results: Methodological improvements are presented which comprise a simpler extraction procedure, the use of phosphatidylbutanol as internal standard and a new algorithm for evaluation of unknown samples. It is further demonstrated that equal test results are obtained with blood collected in standard test tubes with EDTA as with the previously used heparinized test tubes. The PEth content in blood samples is stable for three weeks in the refrigerator.

Conclusion: Methodological changes make the method more suitable for routine laboratory use, lower the limit of quantification (LOQ) and improve precision.

Implications of this study

The authors determined that the anti-coagulant used in the collection tube did not affect PEth levels.

Phosphatidylethanol (PEth) Concentrations in Blood Are Correlated to Reported Alcohol Intake in Alcohol-Dependent Patients

ABSTRACT

Aims: Phosphatidylethanol (PEth) is an abnormal phospholipid formed only in the presence of ethanol by the enzyme phospholipase D. PEth in blood is a promising new marker for ethanol abuse. None of the biological markers used at the present time is sensitive and specific enough for the diagnosis of alcoholism.

Methods: The most frequently used alcohol markers [carbohydrate deficient transferrin (CDT), gamma-glutamyltransferase (GGT), and mean corpuscular volume (MCV)] were studied together with PEth in actively drinking alcohol-dependent patients (inpatients and outpatients), with regard to correlation to ethanol intake and diagnostic sensitivity of the markers. The relation between the markers was also studied.

Results: PEth, CDT, and GGT correlated to ethanol intake, with the strongest correlation found for PEth. The diagnostic sensitivity for PEth was 99%, and for other markers it varied between 40 and 77%. Only when CDT was combined with GGT was a sensitivity of 94% reached. PEth correlated to CDT and GGT but not to MCV. CDT did not correlate to GGT or MCV.

Conclusions: Blood concentrations of PEth are highly correlated to ethanol intake, and the present results indicate that its diagnostic sensitivity is higher than that for previously established alcohol markers.

Implications of this study

This was the first study that related blood PEth levels to self-report and a battery of indirect biomarkers, demonstrated that PEth blood levels are highly correlated, and demonstrated that PEth is a significant improvement over current alcohol markers.

2007 (1)

Phosphatidylethanol as a Sensitive And Specific Biomarker - Comparison With Gamma-Glutamyl Transpeptidase, Mean Corpuscular Volume And Carbohydrate-Deficient Transferrin

ABSTRACT

Phosphatidylethanol (PEth), a direct ethanol metabolite, is detectable in blood for more than 2 weeks after sustained ethanol intake. Our aim was to assess the usefulness of PEth [comparing sensitivity, specificity and the area under the curve (AUC)] as compared with carbohydrate-deficient transferrin (CDT), gamma-glutamyl transpeptidase (GGT) and mean corpuscular volume (MCV), calculating the results from sober patients against those from alcohol-dependent patients during withdrawal. Fifty-six alcohol-dependent patients (ICD-10 F 10.25) in detoxification, age 43 years, GGT 81 U/l, MCV 96.4 fl, %CDT 4.2, 1400 g ethanol intake in the last 7 days (median), were included in the study. Over the time of 1 year, 52 samples from 35 sober forensic psychiatric addicted in-patients [age 34 years, GGT 16 U/l, MCV 91 fl, CDT 0.5 (median)] in a closed ward were drawn and used for comparison . PEth was measured in heparinized whole blood with a high-performance liquid chromatography method. GGT, MCV and %CDT were measured using routine methods. A receiver operating characteristic curve analysis was carried out, with 'current drinking status' (sober/ drinking) as the state variable and PEth, MCV, GGT and CDT as test variables. The resulting AUC was 0.974 (P < 0.0001, confidence interval 0.932–1.016) for PEth. At a cut-off of 0.36 µmol/l, the sensitivity was 94.5% and specificity 100%. The AUC for CDT, GGT and MCV were 0.931, 0.894 and 0.883, respectively. A significant Spearman's rank correlation was found between PEth and GGT (r = 0.739), CDT (r = 0.643), MVC (r = 0.639) and grams of ethanol consumed in the last 7 days (r = 0.802). Our data suggest that PEth has potential to be a sensitive and specific biomarker, having been found in previous studies to indicate longer lasting intake of higher amounts of alcohol.

Implications of this study

This study compared PEth levels in alcohol-dependent patients during withdrawal and treatment and compared them to a battery of indirect markers demonstrating excellent sensitivity and specificity for PEth.

Selective Detection of Phosphatidylethanol Homologues in Blood as Biomarkers For Alcohol Consumption by LC-ESI-MS/MS

ABSTRACT

A new validated method for the quantitation of the abnormal phospholipid phosphatidylethanol (PEth) – a biomarker for ethanol uptake – has been developed by LC-ESI-MS/MS following miniaturised organic solvent extraction and reversed phase chromatography with phosphatidylbutanol (PBut) as internal standard. PEth homologues with two fatty acid substituents – PEth 18 : 1/18 : 1, PEth 16 : 0/16 : 0 – were determined in post-mortem blood collected from heavy drinkers at autopsy and also in whole blood samples from a volunteer after a single 60 g-dose of ethanol. Furthermore, PEth 18 : 1/16 : 0 or its isobaric isomer PEth – 16 : 0/18 : 1 was detected. In comparison to previous high-performance liquid chromatography (HPLC) methods with evaporative light scattering detection (ELSD), the LC-MS/MS-method is more sensitive – with a limit of detection below 20 ng/ml – and more selective for single PEth homologues, while ELSD has been used for detection of the sum of PEth homologues with approximately 10 times less sensitivity. LC-MS/MS enables monitoring of PEth homologues as biomarkers for harmful and prolonged alcohol consumption as with HPLC/ELSD earlier, where PEth is measurable in blood only after more than 50 g ethanol daily intake for more than 2 weeks. Because of its higher sensitivity, there is a potential to detect single heavy drinking by LC-MS/MS, when PEth is formed in very low concentrations. This opens a new field of application of PEth to uncover single or multiple heavy drinking at a lower frequency and with a larger window of detection in blood than before by HPLC/ELSD or by use of other direct markers, e.g. ethyl glucuronide or ethyl sulfate.

Implications of this study

This study demonstrated the superior sensitivity and specificity of LCMSMS over HPLC-ELSD detection of PEth for the detection of risky drinking behavior.

Molecular Species of The Alcohol Biomarker Phosphatidylethanol in Human Blood Measured by LC-MS

ABSTRACT

Background: The alcohol biomarker phosphatidylethanol (PEth) comprises a group of ethanol derived phospholipids formed from phosphatidylcholine by phospholipase D. The PEth molecular species have a common phosphoethanol head group onto which 2 fatty acid moieties are attached. We developed an electrospray ionization (ESI) LC-MS method for qualitative and quantitative measurement of different PEth species in human blood.

Methods: We subjected a total lipid extract of whole blood to HPLC gradient separation on a C4 column and performed LC-ESI-MS analysis using selected ion monitoring of deprotonated molecules for the PEth species and phosphatidylpropanol (internal standard). Identification of individual PEth species was based on ESI–tandem mass spectrometry (MS/MS) analysis of product ions.

Results: The fatty acid moieties were the major product ions of PEth, based on comparison with PEth-16:0/16:0, 18:1/18:1, and 16:0/18:1 reference material. For LC-MS analysis of different PEth species in blood, we used a calibration curve covering 0.2–7.0 µmol/L PEth-16:0/18:1. The lower limit of quantitation of the method was <0.1 µmol/L, and intra- and interassay CVs were <9% and <11%. In blood samples collected from 38 alcohol patients, the total PEth concentration ranged between 0.1 and 21.7 µmol/L (mean 8.9). PEth- 16:0/18:1 and 16:0/18:2 were the predominant molecular species, accounting for approximately 37% and 25%, respectively, of total PEth. PEth-16:0/20:4 and mixtures of 18:1/18:1 plus 18:0/18:2 (not separated using selected ion monitoring because of identical molecular masses) and 16:0/20:3 plus 18:1/18.2 made up approximately 13%, 12%, and 8%.

Conclusions: This LC-MS method allows simultaneous qualitative and quantitative measurement of several PEth molecular species in whole blood samples.

Implications of this study

This was the first published method using LCMSMS monitoring the individual species of PEth.

ABSTRACT

Aims: The goal of this preliminary study was to evaluate the relationship between blood phosphatidylethanol (PEth) and recent drinking in patients with liver disease and hypertension.

Methods: Twenty-one patients with liver disease and 21 patients with essential hypertension were recruited at an academic medical center. Alcohol consumption was estimated using validated self-report methods, and blood PEth was measured by HPLC-MS/MS at a contracted laboratory. Nonparametric comparisons were made between abstainers/light drinkers, moderate drinkers consuming between 1 and 3 drinks per day, and those drinking above this level. Regression methods were used to estimate the effects of liver disease, gender, and age on the relationship between PEth and alcohol use, and to estimate the strength of the linear relationship between PEth and drinking.

Results: PEth differed significantly between the three drinking groups (P < 0.001). The relationship between PEth and alcohol did not differ between hypertension and liver disease patients (P = 0.696), nor by gender and age. While there was substantial variability between subjects in the PEth concentration given a similar level of reported drinking, the amount of ethanol consumed was strongly associated with the PEth concentration (P < 0.001).

Conclusion: Results support PEth measurement by HPLC-MS/MS as a promising marker of past 1- to 2-week moderate to heavy alcohol consumption in patients with and without liver disease. PEth appears useful for differentiating abstinence or light drinking from moderate to heavy consumption, but may have limited utility for differentiating moderate from heavy alcohol use.

Implications of this study

This study demonstrated that liver disease did not affect the levels of PEth and further suggested that PEth may be a suitable biomarker for monitoring chronic disease management, such as hypertension, liver disease, diabetes, and gastro-enteritis.

Estimating Driver Risk Using Alcohol Biomarkers, Interlock Blood Alcohol Concentration Tests And Psychometric Assessments: Initial Descriptives

ABSTRACT

Aim: To identify alcohol biomarker and psychometric measures that relate to drivers' blood alcohol concentration (BAC) patterns from ignition interlock devices (IIDs).

Design, setting, participants, measurements: In Alberta, Canada, 534 drivers, convicted of driving under the influence of alcohol (DUI), installed IIDs and agreed to participate in a research study. IID BAC tests are an established proxy for predicting future DUI convictions. Three risk groups were defined by rates of failed BAC tests. Program entry and follow-up blood samples (n = 302, 171) were used to measure phosphatidyl ethanol (PETH), carbohydrate deficient transferrin (%CDT), gamma glutamyltransferase (GGT) and other biomarkers. Program entry urine (n = 130) was analyzed for ethyl glucuronide (ETG) and ethyl sulphate (ETS). Entry hair samples were tested for fatty acid ethyl esters (FAEE) (n = 92) and ETG (n = 146). Psychometric measures included the DSM-4 Diagnostic Interview Schedule Alcohol Module, Alcohol Use Disorders Identification Test (AUDIT), the time-line follow-back (TLFB), the Drinker Inventory of Consequences (DRINC) and the Temptation and Restraint Inventory (TRI).

Findings: Except for FAEE, all alcohol biomarkers were related significantly to the interlock BAC test profiles; higher marker levels predicted higher rates of interlock BAC test failures. PETH, the strongest with an overall analysis of variance F ratio of 35.5, had significant correlations with all nine of the other alcohol biomarkers and with 16 of 19 psychometric variables. Urine ETG and ETS were correlated strongly with the IID BAC tests.

Conclusions: The findings suggest that several alcohol biomarkers and assessments could play an important role in the prediction and control of driver alcohol risk when re-licensing.

Implications of this study

A very large cohort of DUI offenders were monitored with a variety of direct and indirect biomarkers and a battery of self-report questionnaires. Coupled with their driver interlock data, the study demonstrated that PEth was the most predictive marker of relapse.

Phosphatidylethanol And Alcohol Consumption in Reproductive Age Women

ABSTRACT

Background: Fetal alcohol disorders are preventable, but self-reported alcohol consumption can be misleading and impede effective treatment. Biomarkers represent an alternative method for assessing alcohol use, and this study evaluated the relationship between blood phosphatidylethanol (PEth) and alcohol use in a sample of reproductive age women.

Methods: Alcohol use was estimated by validated self-report methods in 80 nonpregnant women ages 18 to 35. PEth was measured by a contracted laboratory using a liquid chromatography-tandem mass spectrometry assay. Regression methods appropriate for the distribution PEth were used to define its relationship to alcohol consumption during the prior 2 weeks and explore the effects of drinking patterns on this association. Receiver operating characteristic analysis was used to estimate the sensitivity of PEth for various drinking levels at 95% specific cutoffs.

Results: PEth had a positive linear association with grams of alcohol consumed (p < 0.001), and was detectable in 93% of subjects consuming an average of 2 or more drinks per day. The relationship between total alcohol consumption and PEth may be stronger in women with recent heavy drinking days. The relationship between drinking and PEth varied considerably between individuals, and sensitivity for a certain amount of drinking was low at a highly specific cutoff concentration.

Conclusions: PEth is a highly sensitive indicator of moderate and heavy alcohol consumption in reproductive age women and may complement the use of self-report alcohol screens when additional objective markers of alcohol use are desirable. However, choosing a highly valid cutoff concentration for PEth to differentiate various levels of alcohol consumption may not be feasible.

Implications of this study

This study used PEth blood levels from women of reproductive age and compared the results to validated self-reports demonstrating a positive linear relationship between PEth levels and grams of ethanol consumed.

Phosphatidylethanol: Normalization During Detoxification, Gender Aspects And Correlation With Other Biomarkers And Self-reports

Wurst, F. M., Thon, N., Aradottir, S., Hartmann, S., Wiesbeck, G. A., Lesch, O., & Alling, C.

ABSTRACT

Phosphatidylethanol (PEth) is a direct ethanol metabolite, and has recently attracted attention as biomarker of ethanol intake. The aims of the current study are: (1) to characterize the normalization time of PEth in larger samples than previously conducted; (2) to elucidate potential gender differences; and (3) to report the correlation of PEth with other biomarkers and self-reported alcohol consumption. Fifty-seven alcohol-dependent patients (ICD 10 F 10.25; 9 females, 48 males) entering medical detoxification at three study sites were enrolled. The study sample was comprised of 48 males and 9 females, with mean age 43.5. Mean gamma glutamyl transpeptidase (GGT) was 209.61 U/l, average mean corpuscular volume (MCV) was 97.35 fl, mean carbohydrate deficient transferrin (%CDT) was 8.68, and mean total ethanol intake in the last 7 days was 1653 g. PEth was measured in heparinized whole blood with a high-pressure liquid chromatography method, while GGT, MCV and %CDT were measured using routine methods. PEth levels at day 1 of detoxification ranged between 0.63 and 26.95 mmol/l (6.22 mean, 4.70 median, SD 4.97). There were no false negatives at day 1. Sensitivities for the other biomarkers were 40.4% for MCV, 73.1% for GGT and 69.2% for %CDT, respectively. No gender differences were found for PEth levels at any time point. Our data suggest that PEth is (1) a suitable intermediate term marker of ethanol intake in both sexes; and (2) sensitivity is extraordinary high in alcohol dependent patients. The results add further evidence to the data that suggest that PEth has potential as a candidate for a sensitive and specific biomarker, which reflects longer-lasting intake of higher amounts of alcohol and seemingly has the above mentioned certain advantages over traditional biomarkers.

Implications of this study

This study evaluated 57 alcohol dependent patients entering a detoxification facility. The study found that that sensitivity of PEth on day 1 of detoxification was 100% and the sensitivity of MCV, GGT, and CDT were 40.4%, 73.1% and 69.2%, respectively. The positivity rate for patients undergoing detoxification was 100% on day 1, 92.5% on day 7, 76% on day 14 and 64.3% on day 28. The relapse of two patients was identified because of an increasing PEth level after transferring to outpatient status and was confirmed with elevated ETG urine levels. There were not any observable gender differences in the detection of PEth.

The Detection of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol in Human Dried Blood Spots

ABSTRACT

Phosphatidylethanol, a series of abnormal phospholipids formed in the presence of ethanol and phospholipase D, has gained popularity as a long-term biomarker of ethanol ingestion. A liquid chromatography tandem mass spectrometric method for a specific, prevalent isomer, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol, was developed and validated using dried blood spots. Dried blood spots offer numerous advantages over venipuncture including reduced costs, invasiveness and discomfort. Dried blood spots were prepared from authentic whole blood specimens that had been tested using a previously published procedure. Comparison of the results from the two assays demonstrated excellent correlation. The data suggest that dried blood spots may be a useful tool for the detection of alcohol abuse and abstinence monitoring.

Implications of the study

This was the first published method to identify PEth using dried blood spots.

LC-MS/MS Analysis of Phosphatidylethanol in Dried Blood Spots Versus Conventional Blood Specimens

ABSTRACT

Phosphatidylethanol (PEth), which is formed extrahepatically by the action of phospholipase D on phosphatidylcholine in the presence of ethanol, has been suggested as a promising marker of alcohol misuse. Analysis of dried blood spots (DBS) is particularly advantageous for the determination of delicate analytes such as PEth. Therefore, measurement of PEth species (18:1/18:1, 16:0/18:1) in DBS versus whole blood was performed to ascertain whether respective results are directly comparable. Samples were obtained from subjects (n = 40) undergoing alcohol detoxification treatment. Analysis involved liquid–liquid extraction from both, DBS and whole blood (100 μL, respectively), with phosphatidylpropanol as the internal standard. Extracts were subjected to LC gradient separation using multiple reaction monitoring of deprotonated molecules. Results from measurements of corresponding DBS and whole blood specimens were compared by estimating the respective mean values and by a Bland and Altman analysis. Concentrations of PEth 18:1/18:1 ranged from 46.1 to 3,360 ng/mL in whole blood (mean, 461.7 ng/mL) and from 35.8 to 3,360 ng/mL in DBS (mean, 457.6 ng/mL); for PEth 16:0/18:1, concentrations were from 900 to 213,000 ng/mL (mean, 23,375 ng/mL) and 922– 213,000 ng/mL (mean, 23,470 ng/mL) in blood and DBS, respectively. Estimated mean differences were −4.3 ng/mL for PEth 18:1/18:1 and 95.8 ng/mL for PEth 16:0/18:1. The Bland–Altman plot of both PEth species showed that the variation around the mean difference was similar all through the range of measured values and that all differences except one were within the limits of agreement. It could be shown that the determination of PEth species in DBS is as reliable as in whole blood samples. This assay may facilitate monitoring of alcohol misuse.

Implications of this study

An independent laboratory in Europe confirmed the findings of USDTL. Blood spots are a valuable tool for measuring PEth.

Detection of Phosphatidylethanol (PEth) in The Blood of Drivers in an Alcohol Ignition Interlock Program

ABSTRACT

Objective: The rate of failed interlock blood alcohol content (BAC) tests is a strong predictor of recidivism post-interlock and a partial proxy for alcohol use. Alcohol biomarkers measured at the start of an interlock program are known to correlate well with rates of failed BAC tests over months of interlock use. This study evaluates 2 methods of measuring low blood levels of the biomarker phosphatidylethanol (PEth). PEth is a 100 percent alcohol-specific biomarker and strongly intercorrelated with several independent indicators of drinking driving risk, including 8 other biomarkers, 3 psychometric assessments, and the rate of failed interlock BAC tests during many months of interlock use. Does a more sensitive method of measuring PEth at program entry detect drinking even among those who subsequently log no failed interlock tests?

Methods: In a sample of 281 driver blood samples, PEth was measured by both high-performance liquid chromatography (HPLC) and liquid chromatography tandem mass spectrometry (LCMSMS) in order to compare sensitivity and accuracy. The average rate of failed interlock BAC tests was the criterion measure for marker sensitivity. LCMSMS, calibrated to detect low levels of drinking as a possible measure of abstinence violation, was judged relative to the standard HPLC assay for PEth measured up to 4 μmol/L.

Results: The 2 methods showed a good quantitative relationship ($r^2 > .86$). LCMSMS detected positive PEth levels in samples that were below the limit of detection of the HPLC method. PEth measured by LCMSMS was positive for a higher proportion of driving under the influence (DUI) offenders who logged zero failed interlock BAC tests than were detected by HPLC.

Conclusion: Although HPLC is the widely used standard for measuring PEth in clinical alcoholism samples, the LCMSMS method, when calibrated to detect trace amounts of the major component of PEth, can detect abstinence levels of alcohol near zero intake and still correlate strongly with other indicators related to alcohol use and road safety.

Implications of this study

This study demonstrated the strong positive correlation of the detection of PEth using the evaporative light scattering detector and the tandem mass spectrometer method. It further demonstrated that the tandem mass spectrometer method was more sensitive and more suitable for abstinence detection.

Formation of Phosphatidylethanol and Its Subsequent Elimination during an Extensive Drinking Experiment Over 5 Days

ABSTRACT

Background: For almost 30 years, phosphatidylethanol (PEth) has been known as a direct marker of alcohol consumption. This marker stands for consumption in high amounts and for a longer time period, but it has been also detected after 1 high single intake of ethanol (EtOH). The aim of this study was to obtain further information about the formation and elimination of PEth 16:0/18:1 by simulating extensive drinking.

Methods: After 3 weeks of alcohol abstinence, 11 test persons drank an amount of EtOH leading to an estimated blood ethanol concentration of 1 g/kg on each of 5 successive days. After the drinking episode, they stayed abstinent for 16 days with regular blood sampling. PEth 16:0/18:1 analysis was performed using liquid chromatography-tandem mass spectrometry (high-performance liquid chromatography 1100 system and Q-Trap 2000 triple quadrupole linear ion trap mass spectrometer. Values of blood alcohol were obtained using a standardized method with headspace gas chromatography flame ionization detector.

Results: Maximum measured concentrations of EtOH were 0.99 to 1.83 g/kg (mean 1.32 g/kg). These values were reached 1 to 3 hours after the start of drinking (mean 1.9 hours). For comparison, 10 of 11 volunteers had detectable PEth 16:0/18:1 values 1 hour after the start of drinking, ranging from 45 to 138 ng/ml PEth 16:0/18:1. Over the following days, concentrations of PEth 16:0/18:1 increased continuously and reached the maximum concentrations of 74 to 237 ng/ml between days 3 and 6.

Conclusions: This drinking experiment led to measurable PEth concentrations. However, PEth 16:0/18:1 concentrations stayed rather low compared with those of alcohol abusers from previous studies.

Implications of this study

This study evaluated the quantity of alcohol required to produce positive PEth values. The subjects were not abusers or dependents as in previous studies. Instead, this study used a group of social drinkers that were able to achieve a negative baseline reading prior to initiating the measured dose study that lasted five days, followed by 16 days of abstinence. The dosing was proportional to body size in an attempt to achieve a blood alcohol concentration of 0.100%. This experiment demonstrated that individuals must achieve significant blood alcohol concentrations to produce positive blood PEth values.

Phosphatidylethanol (PEth) as a Biomarker of Alcohol Consumption in HIV-Positive Patients in Sub-Saharan Africa

ABSTRACT

Background: Alcohol is heavily consumed in sub-Saharan Africa and affects HIV transmission and treatment and is difficult to measure. Our goal was to examine the test characteristics of a direct metabolite of alcohol consumption, phosphatidylethanol (PEth).

Methods: Persons infected with HIV were recruited from a large HIV clinic in southwestern Uganda. We conducted surveys and breath alcohol concentration (BRAC) testing at 21 daily home or drinking establishment visits, and blood was collected on day 21 (n = 77). PEth in whole blood was compared with prior 7-, 14-, and 21-day alcohol consumption.

Results: (i) The receiver operator characteristic area under the curve (ROCAUC) was highest for PEth versus any consumption over the prior 21 days (0.92; 95% confidence interval [CI]: 0.86 to 0.97). The sensitivity for any detectable PEth was 88.0% (95% CI: 76.0 to 95.6) and the specificity was 88.5% (95% CI: 69.8 to 97.6). (ii) The ROC-AUC of PEth versus any 21-day alcohol consumption did not vary with age, body mass index, CD4 cell count, hepatitis B virus infection, and antiretroviral therapy status, but was higher for men compared with women (p = 0.03). (iii) PEth measurements were correlated with several measures of alcohol consumption, including number of drinking days in the prior 21 days (Spearman r = 0.74, p < 0.001) and BRAC (r = 0.75, p < 0.001).

Conclusions: The data add support to the body of evidence for PEth as a useful marker of alcohol consumption with high ROC-AUC, sensitivity, and specificity. Future studies should further address the period and level of alcohol consumption for which PEth is detectable.

Implications of this study

This study demonstrated a high degree of correlation of blood PEth concentrations, breath alcohol, and number of self-reported drinks in an unhealthy population (HIV infected). Other alcohol biomarkers may be affected by poor health of the liver or kidneys but since PEth production is ubiquitous the levels appear to be unaffected by poor health.

Monitoring of the Alcohol Biomarkers PEth, CDT and EtG/EtS in an Outpatient Treatment Setting

ABSTRACT

Aims: To compare the sensitivity of whole blood phosphatidylethanol (PEth) with serum carbohydrate-deficient transferrin (CDT) as biomarkers of current regular alcohol consumption, during outpatient treatment for alcohol related problems. Urinary ethyl glucuronide (EtG) and ethyl sulfate (EtS), and clinical assessment, were used as complementary estimates of relapse to drinking.

Methods: Biomarker results for 29 men and 11 women (aged 20–73 years) undergoing voluntary outpatient treatment for harmful alcohol use or dependence were utilized for this evaluation. In connection with visits to the unit, blood and/or urine were sampled for measurement of PEth, EtG and EtS (by liquid chromatography-mass spectrometry), and CDT (% di-sialotransferrin, by high-pressure liquid chromatography).

Results: The comparison included 326 whole blood, 319 serum (1–82 samples/patient) and 654 urine samples (1–178 samples/patient) collected over ~2 years. At the initial assessment, the total PEth value ranged between 0 and 16.5 µmol/l (mean 2.6) with 70% being above the quantification limit (0.1 µmol/l) and 55% above the reference interval (0.7 µmol/l). Initial CDT values were 0.87–6.9% (mean 2.1) with 35% above the applied reference interval (1.7%). At the final sampling (treatment period up to 21 months), the total PEth value had decreased to 0–5.9 µmol/l (mean 0.6; P = 0.0004) and CDT to 0.87–3.3% (mean 1.3; P = 0.0030). Relapses were detected by PEth alone (43% of cases), by PEth and CDT (38%) and the remainder by EtG/EtS.

Conclusion: PEth was the most sensitive biomarker of current regular alcohol consumption. PEth-16:0/18:1, usually being the major sub-form, was as sensitive as total PEth. PEth, CDT and EtG/EtS are useful complementary tools for objective identification of current drinking and relapse detection.

Implications of this study

This study evaluated the effectiveness of the alcohol biomarkers blood PEth, serum CDT, and urine EtG/EtS. The authors concluded that PEth was more suitable for the detection of relapse that CDT and lacked the over-sensitivity of urine EtG/EtS. Based on these results, the outpatient center for the drunk-drivers program is initiating the use of PEth to detect relapse as an alternative to ankle bracelet strategies.

Direct Alcohol Biomarkers as Tools to Guide Meaningful Interventions While Monitoring Repeat Intoxicated Drivers in Kenosha County

Bean, P., Brown, G., & Lewis, D. (2012). Research Society on Alcoholism National Conference. Poster Presentation. San Francisco.

ABSTRACT

The goal of this study was to evaluate the performance of two direct alcohol biomarkers as tools to monitor drinking behavior in repeat intoxicated drivers during the 12-month period of their required driver's safety plans. When relapses were detected, the biomarker information was used to conduct a brief intervention to motivate a change in drinking behavior. The study analyzed 32 drivers who came to the Hope Council on Alcohol and Other Drug Abuse for a court-mandated assessment after having been arrested for driving under the influence (DUI). All drivers had at least 3 DUI offenses in their lifetimes, and 50% had a BAC above 0.2% at the time of the arrest. The biomarkers used were ethyl glucuronide (EtG) in fingernails and phosphatidylethanol (PEth) in dried blood spots. Sample collection was done on site by collecting fingernail clippings and blood spots. Samples were sent to USDTL to determine EtG and PEth levels using liquid chromatography and tandem mass spectrometry. Each driver was tested at the assessment interview (baseline) and at 3 and 8 months follow-up. The analysis consisted of classifying drivers into four main groups: 1) Abstainers were drivers who tested biomarker negative at baseline and follow-up; 2) Reducers were drivers who tested biomarker positive at baseline followed by a continuous decline in biomarker values at the follow-up periods; 3) Relapsers were drivers who showed an increase in biomarker levels at any of the monitoring periods; and 4) Non-compliant were drivers who did not comply with biomarker testing at follow-up. The results showed that 19% (6/32) of drivers classified as abstainers; 59% (19/32) of drivers reduced their drinking from baseline to follow-up; 6% (2/32) suffered a relapse; and, 16% (5/32) became non-complaint. Since the reducers represent the largest group in this ongoing analysis, this study supports the value of brief interventions in changing the drinking behavior of repeat intoxicated drivers during follow-up. After drivers discussed the biomarker data with their assessors, most of them experienced a decrease in drinking behavior at follow-up. By using direct biomarkers, Kenosha officials can now more accurately flag the "extreme high risk" drivers, conduct more meaningful interventions, and refer them to more frequent monitoring. Developing evidence-based practices is helping Kenosha County allocate resources more effectively and thus increasing public safety by attempting to decrease drunk driving.

Implications of this study

Kenosha County in Wisconsin has found the use of PEth as a valuable tool to monitor for alcohol relapse among repeat drunk driver offenders enrolled in treatment, specifically to provide rapid feedback to participants to return them to abstinence.

Phosphatidylethanol in Blood as a Marker of Chronic Alcohol Use: A Systematic Review And Meta-Analysis

ABSTRACT

The present paper aims at a systematic review of the current knowledge on phosphatidylethanol (PEth) in blood as a direct marker of chronic alcohol use and abuse. In March 2012, the search through "MeSH" and "free-text" protocols in the databases Medline/PubMed, SCOPUS, Web of Science, and Ovid/Embase, combining the terms phosphatidylethanol and alcohol, provided 444 records, 58 of which fulfilled the inclusion criteria and were used to summarize the current evidence on the formation, distribution and degradation of PEth in human blood: (1), the presence and distribution of different PEth molecular species (2), the most diffused analytical methods devoted to PEth identification and quantization (3), the clinical efficiency of total PEth quantification as a marker of chronic excessive drinking (4), and the potential utility of this marker for identifying binge drinking behaviors (5). Twelve papers were included in the meta-analysis and the mean (M) and 95% confidence interval (CI) of total PEth concentrations in social drinkers (DAI $\leq$ 60 g/die; M = 0.288 μM; CI 0.208-0.367 μM) and heavy drinkers (DAI > 60 g/die; M = 3.897 μM; CI 2.404-5.391 μM) were calculated. The present analysis demonstrates a good clinical efficiency of PEth for detecting chronic heavy drinking.

Implications of this review

This study presented a comprehensive, systematic review of the existing PEth research, and affirmed the usefulness of PEth as a biomarker of chronic heavy drinking. By presenting a cohesive picture of the current state of PEth research, the authors were also able to provide direction for future PEth research. Most notably, the authors showed that with more in depth research, PEth may have potential as a biomarker to distinguish between different levels of alcohol consumption, e.g. no, moderate, heavy, or risky consumption.

Stability of Phosphatidylethanol Species in Spiked And Authentic While Blood And Matching Dried Blood Spots

ABSTRACT

Background: Phosphatidylethanol (PEth) is currently under investigation as a highly sensitive and specific marker of alcohol misuse. As its stability in blood samples has not systematically been investigated, a study was performed to determine the stability of major PEth species in spiked and authentic whole blood and also in matching dried blood spots (DBS) at different conditions.

Methods: To PEth-free blood from teetotalers, low and high concentrations of two major PEth (18:1/18:1 and 16:0/18:1) species were added chosen on the basis of concentrations determined from authentic samples which were collected from the subjects undergoing alcohol detoxification treatment. Effects of sampling (EDTA or heparinized tubes), temperature, and time ($\leq$30 days) were investigated. Processed samples (two at each condition, respectively) were subjected to LC gradient separation using multiple reaction monitoring. Stability was assessed using the critical difference or a periodic analysis result that was within 15 % of the initial concentration. Reaction kinetics of degradation was investigated with rate constants being checked for an Arrhenius relationship.

Results: PEth was stable in dried blood spot (DBS) stored either at room temperature or frozen, whereas it was not stable in whole blood except in samples stored at -80°C. Activation energies increased in the following order: spiked heparinized blood < spiked EDTA blood < authentic EDTA blood.

Conclusions: PEth is a labile analyte which is predominantly degraded by hydrolysis. Only at -80°C, stability in whole blood can be ascertained, and analysis should be performed within 30 days. EDTA should be preferred over heparin as an additive. DBS is able to stabilize PEth thus partly resolving pre-analytical difficulties of PEth measurement.

Implications of this study

This study demonstrated the stability of PEth in dried blood spots (DBS), and showed that DBS sampling overcomes the limitations of whole blood collection. The authors showed that *ex vivo* PEth formation and degradation do not occur when DBS are used for PEth testing. From this research, DBS seem to be the preferred sample collection method for PEth testing.

The Detection of 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanol and Ethyl Glucuronide in Human Umbilical Cord

ABSTRACT

In utero exposure to ethanol continues to be a significant public health issue and neonatal healthcare professionals are in need of objective means to identify exposed newborns. The aim of this study was to fully validate two methods for the detection of two direct alcohol biomarkers, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanol (POPE) and ethyl glucuronide (EtG), in umbilical cord and apply the assays to a group of authentic specimens. The limits of detections were 2 and 1 ng/g for POPE and ETG and the limits of quantitation were 4 and 3 ng/g, respectively. Inter and intra-day precision and accuracy measurements were within 15%. The assays were applied to 308 authentic specimens where we detected POPE in five (1.6%) specimens and EtG in twelve (3.9%) specimens. The mean concentrations were 11.4 ng/g ± 9.4 ng/g and 127.2 ± 227.7 ng/g for POPE and EtG, respectively. This study suggested that umbilical cord was a suitable specimen type for the identification of newborns exposed to ethanol in the womb and the prevalence of POPE and EtG detected in umbilical cord were consistent with the prevalence of self-reported binge drinking reported by the National Birth Defect Prevention Study (NBDPS) and Behavioral Risk Factor Surveillance System (BRFSS). Further studies are required to fully describe the association between the observed concentrations of POPE and EtG in umbilical cord to the level of maternal consumption of ethanol.

Implications of the study

This USDTL study was the first to detect PEth (here called POPE) in newborn umbilical cord tissue, and demonstrate a fully validated methodology for umbilical cord PEth testing.

The Feasibility And Cost of Neonatal Screening For Prenatal Alcohol Exposure by Measuring Phosphatidylethanol in Dried Blood Spots

Bakhireva, L., Savich, R.D., Raisch, D.W., Cano, S., Annett, R.D., Leeman, L., Garg, M., Goff, C., & Savage, D.D.

ABSTRACT

Background: Accurate confirmation of prenatal alcohol exposure (PAE) is required as a diagnostic criterion for the majority of children adversely affected by PAE who do not manifest the physical features associated with fetal alcohol syndrome. A number of ethanol biomarkers have been used to assess PAE, often with suboptimal results. The purpose of this study was to evaluate the feasibility and cost of PAE screening in newborns by measuring phosphatidylethanol (PEth) in dried blood spot (DBS) cards.

Methods: The feasibility of collecting an additional DBS card during routine newborn screening and the background prevalence of PAE were evaluated in a de-identified sample of newborn children delivered at the University of New Mexico Hospital. Electronic orders to collect DBS cards from newborns who continue to bleed after the routine newborn screen, glucose, or hematocrit testing were initiated for all infants delivered during a 4-week time frame. Specimens were sent to a contract laboratory for PEth analysis by liquid chromatography-tandem mass spectrometry. A cost analysis was conducted to compare the cost of PAE screening by PEth in DBS versus PEth in conventional blood specimens and by meconium fatty acid ethyl esters.

Results: From 230 collected cards, 201 (87.4%) had at least 1 full blood spot (amount sufficient for PEth analysis), and 6.5% had PEth >20 ng/ml indicative of potential PAE in late pregnancy. PAE screening by PEth in DBS is logistically simpler and less expensive compared with 2 other screening approaches.

Conclusions: These results indicate that screening for PAE in DBS cards is a feasible procedure and that a majority of infants have enough blood after the routine heel prick to fill an additional card. Moreover, screening by PEth analysis from DBS cards is cost-efficient. The acceptability of such screening by parents and corresponding ethical issues remain to be investigated.

Implications of this study

This study demonstrated the ease and cost-effectiveness of screening newborns for prenatal alcohol exposure (PAE) using PEth testing in dried blood spots as an objective measure of ethanol exposure. The authors also identified a much higher prevalence of PAE in their study population than the reported national average.

Phosphatidylethanol: The Potential Role in Further Evaluating Low Positive Urinary Ethyl Glucuronide and Ethyl Sulfate Results

ABSTRACT

Background: Whereas urinary ethyl glucuronide (EtG) levels above 1,000 ng/ml reflect with a high probability ethanol (EtOH) consumption, levels below this cutoff are difficult to interpret as both extraneous (nonbeverage) EtOH exposure, recent drinking, and more distant high EtOH intake (several days ago) might yield similar results. This might be of particular relevance in medico-legal cases. To overcome this dilemma, phosphatidylethanol (PEth) might be a promising marker, because blood PEth is only positive following significant alcohol use. The aim of our study was therefore to employ PEth as a marker to differentiate between the different conditions.

Methods: Subjects included were 252 participants in monitoring with the Alabama Physician Health Program. All subjects testing positive for EtG and/or ethyl sulfate (EtS) who denied drinking after routine supportive confrontation were subject to information about PEth testing. If they still denied drinking, PEth testing was performed and the result communicated. EtG, EtS, and PEth testing was performed in a commercial laboratory using liquid chromatography tandem mass spectrometry methods.

Results: Of a total of 18 subjects who tested positive for EtG and/or EtS, 10 denied drinking. Of the 7 who denied drinking after PEth explanation, in 5 cases, their claim was supported by a negative PEth result. In 2 cases, a positive PEth result was in contrast to their claim.

Conclusions: PEth results in combination with previous low positive EtG/EtS results allow differentiating between innocent/extraneous exposure and drinking. Negative PEth testing following low positive EtG/EtS results helps to further elucidate the findings and support the claim of the patient of recent alcohol abstinence. Positive PEth testing following positive EtG/EtS results confirms recent drinking.

Implications of this study

The study authors used PEth testing to clarify ethyl glucuronide (EtG) and ethyl sulfate (EtS) testing results in the context of abstinence monitoring for a Physicians Health Program. PEth testing was used to evaluate EtG/EtS positive results during treatment in order to distinguish between alcohol use relapse and accidental/incidental exposure to alcohol (through common products such as hand sanitizer, which can occasionally cause a positive EtG/EtS result). PEth was able to differentiate between the two outcomes.

Time Dependence of Elimination of Different PEth Homologues in Alcoholics in Comparison With Social Drinkers

ABSTRACT

Background: Phosphatidylethanol (PEth) is a direct marker of alcohol consumption, which has been known for almost 30 years. Each PEth molecule carries 2 fatty acids, which differ in chain length and degree of unsaturation. It is formed by means of phospholipase D in the presence of ethanol. Usually, this marker was used by quantification of the PEth homologue 16:0/18:1. The intention of this work was to get more information about the distribution and the quantity of the different PEth homologues.

Methods: Blood samples from 12 alcohol-dependent subjects were collected and analyzed during withdrawal therapy. For comparison, blood from 78 healthy social drinkers was also analyzed. PEth analysis was performed as follows: after liquid–liquid extraction, the homologues were separated on a Luna Phenyl Hexyl column, injected to an HPLC system (1100 system; Agilent) and identified by ESI-MS/MS (QTrap 2000; AB Sciex) using multiple reaction monitoring.

Results: PEth 16:0/18:1 is the major homologue comparing the area ratios of PEth homologues in blood samples from alcoholics. Additional prevalent homologues were PEth 16:0/18:2, 18:0/18:2, and 18:0/18:1. The homologues occurring in blood samples from alcoholics as well as from social drinkers were mostly the same, but differences among their distribution pattern were observed.

Conclusions: In addition to the approach to quantitate the PEth homologue 16:0/18:1, this is a new and alternative proceeding for the differentiation between alcoholics and social drinkers using this alcohol consumption marker.

Implications of this study

This study confirms previous research showing that the palmitoyl/oleoyl (16:0/18:1, POPE) isomer is the most abundant PEth homolog. The authors also demonstrate patterns in PEth homolog abundance/degradation that may, with further research, be useful in identifying how recently a person has consumed alcohol.

Asymptomatic phosphomannose isomerase deficiency (MPI-CDG) initially mistaken for excessive alcohol consumption.

ABSTRACT

Case Report: In a routine company health check-up, a 32-year-old woman presented a highly elevated serum level of carbohydrate-deficient transferrin (CDT), a biomarker for excessive alcohol consumption. The test result (~17% disialotransferrin, reference interval <2.0%; ~3% asialotransferrin, reference 0%) was confirmed by analysis of a second sample, while another alcohol biomarker, phosphatidylethanol (PEth) in whole-blood, was negative. This suggested that her elevated CDT may be unrelated to heavy drinking. The abnormal "type-1" transferrin glycoform pattern indicated a defect in N-glycan assembly occurring in congenital disorders of glycosylation (CDG), a family of rare inherited metabolic disorders. Probing for the underlying enzyme defect(s) using cultured skin fibroblasts demonstrated normal activity of phosphomannomutase, whereas the activity of phosphomannose isomerase (MPI) was reduced (0.64mU/mg protein, reference 2.1-6.9), pointing to CDG of the MPI subtype (formerly called CDG-Ib). The diagnosis was confirmed by sequence analysis of the MPI gene revealing a homozygous missense mutation (c.656G>A) causing replacement of arginine by glutamine (p.R219Q). However, the woman had never experienced any clinical manifestations associated with MPI-CDG. Both parents, being distant relatives, were heterozygous mutation carriers with normal CDT values. Two of three siblings were not affected, whereas one brother was also homozygous for c.656G>A and had a highly elevated CDT and no clinical symptoms.

Conclusion: The finding of MPI-CDG adults without clinical manifestations suggests that this type of the disorder may be underdiagnosed. If asymptomatic MPI-CDG subjects undergo CDT screening, their highly elevated test results may be wrongly interpreted as caused by excessive alcohol consumption.

Implications of this study

Previous studies have demonstrated the high degree of specificity of PEth as a direct alcohol biomarker for identifying alcohol use. This case study not only highlights that specificity against a commonly used indirect alcohol biomarker (CDT), but also illustrates the drawbacks of using indirect biomarkers that can be produced even in the absence of alcohol use. The utility of PEth in distinguishing alcohol use from a physiological health condition is well demonstrated here.

The validity of phosphatidylethanol in dried blood spots of newborns for the identification of prenatal alcohol exposure.

ABSTRACT

Background: Accurate identification of prenatal alcohol exposure (PAE) in the newborn period offers an opportunity for early identification of children at risk of future neurocognitive problems and the implementation of interventional approaches earlier in life. PAE newborn screening by measuring phosphatidylethanol in dried blood spot (PEth-DBS) cards is feasible, logistically easier, and more cost-efficient compared with other biomarkers. However, the sensitivity and specificity of this method have yet to be established.

Methods: This prospective cohort study examined validity of PEth-DBS among 28 infants with PAE and 32 controls relative to maternal self-report and other biomarkers. Pregnant women were recruited from a University of New Mexico clinic and followed to early postpartum period. The composite index, which was based on self-reported measures of alcohol use and allowed to classify subjects into PAE and control groups, was the criterion measure used to estimate sensitivity and specificity of PEth-DBS.

Results: The study included large proportions of patients representing ethnic minorities (7.4% American Indian, 81.7% Hispanic/Latina), low education (54.2% <high school), and unplanned pregnancy (90.0%). No differences in sociodemographic characteristics, smoking or illicit drug use were observed among the study groups. The sensitivity of maternal biomarkers (gamma glutamyltranspeptidase [GGT], % carbohydrate-deficient transferrin [%CDT], urine ethyl glucuronide [UEtG], urine ethyl sulfate [UEtS]) was low (<15%) reflecting a moderate chronic or intermittent binge pattern of drinking in this cohort. PEth-DBS demonstrated 100% specificity and the highest sensitivity (32.1%) compared with other biomarkers. A battery consisting of maternal direct ethanol metabolites (UEtG, UEtS, PEth) and newborn PEth-DBS increased sensitivity to 50% without a substantial drop in specificity (93.8%).

Conclusions: Newborn PEth-DBS is a highly specific biomarker and can facilitate accurate detection of PAE in conjunction with other biomarkers. Minimal invasiveness, ease of storage and transportation of DBS cards, absence of postcollection synthesis, cost savings, and potential integration with routine newborn screening are all unique advantages of this method.

Implications of this study

In this study, the direct alcohol biomarker PEth was compared against several commonly tested indirect alcohol biomarkers for the ability to identify prenatal alcohol exposure. PEth outperformed every indirect biomarker, for combined sensitivity and specificity. This study also highlighted the ease of PEth testing in newborns using dried blood spot sample collection.

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