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Utilizing immunoaffinity chromatography (IAC) cross-reactivity in GC–MS/MS exemplified at the measurement of prostaglandin E_1 in human plasma using prostaglandin E_2-specific IAC columns☆

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ABSTRACT

Immunoaffinity chromatography (IAC) is an elegant and highly efficient method to isolate a particular compound from biological samples for measurement by mass spectrometry coupled to GC, CE, or LC. The utility of IAC for the quantitative determination of several prostaglandins including prostaglandin E_2 (PGE_2) by GC–MS/MS and LC–MS/MS has been demonstrated. The aim of the present work was to test whether the cross-reactivity of the antibody immobilized on an insoluble support can be utilized for the quantitative determination of biomolecules by stable-isotope dilution mass spectrometry. In this communication, we provide evidence that this is indeed possible for prostaglandin E_1 (PGE_1) in human plasma by GC–MS/MS using commercially available Sepharose 4-based IAC columns with immobilized mouse anti-PGE_2 monoclonal antibody with a declared cross-reactivity of about 19% toward PGE_1. Endogenous PGE_1 and the internal standard [3,3′,4,4′-$^2H_4$]-PGE_1 (d_4-PGE_1) externally added to human plasma samples were extracted by IAC, converted to their pentafluorobenzyl ester-methoxime-trimethylsilyl ether derivatives and analyzed by GC–MS/MS in the electron-capture negative-ion chemical ionization mode. Quantification was performed by selected-reaction monitoring of the mass transition m/z 526 → m/z 258 for PGE_1 and m/z 530 → m/z 262 for d_4-PGE_1. By this method we measured PGE_1 concentrations in EDTA plasma samples (1 mL) of six healthy volunteers in the range 10–25 pg/mL (29–72 pM). PGE_1 plasma concentration showed a trend for positive correlation with plasma parameters such as low density lipoprotein (LDL)-cholesterol, total cholesterol and glucose. The method described here provides a novel tool to study the potential link of PGE_1 formation to dyslipidemia, insulin resistance and related metabolic disorders.

1. Introduction

Cyclooxygenase (COX), synonymously used with prostaglandin H synthase (PGHS), peroxidizes free arachidonic acid (AA; 20:4 n-6) to various prostaglandins (PG) including PGE_2 (9-oxo-11α,15*S*-dihydroxy-prosta-5*Z*,13*E*-dien-1-oic acid; Scheme 1) which is subsequently metabolized by enzyme-catalyzed oxidation and reduction reactions. Reduction of the 5*Z* double bond of PGE_2 would yield PGE_1 (9-oxo-11α,15*S*-dihydroxy-prosta-13*E*-dien-1-oic acid; Scheme 1). However, formation of PGE_1 from PGE_2 has not been reported thus far [1,2]. PGE_1 has been reported to be produced from dihomo-gamma-linolenic acid (DGLA; 20:3 ω-6) by the catalytic action of COX [3]. Both PGE_2 and PGE_1 are physiological prostaglandins, occur virtually in all biological fluids and tissues, and possess biological activity. Synthetic PGE_2 (Dinoprostone) and PGE_1 (Alprostadil) are used as drugs. The concentration of PGE_2, PGE_1, and their metabolites from endogenous sources and drugs in plasma of healthy humans is in the lowest pg/mL-range as measured by mass spectrometry (MS) coupled to gas chromatography (GC–MS/MS) [4,5] or liquid chromatography (LC–MS/MS) in combination with the use of stable-isotope labeled analogs (reviewed in Refs. [6,7]).

PGE_2, PGE_1, and other eicosanoids are commonly isolated from biological fluids by liquid-phase extraction (LPE) and/or

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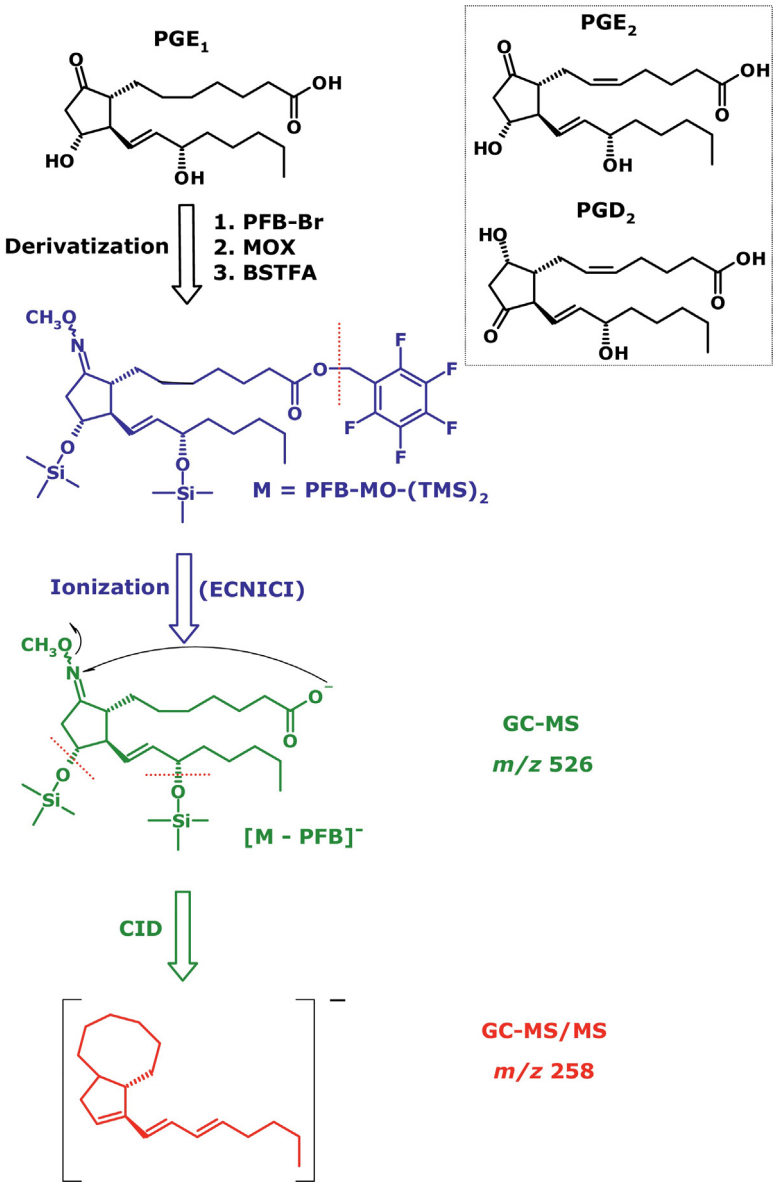

Scheme 1. Schematic of the derivatization steps for PGE_1 and the subsequent analysis by GC–MS/MS. For comparison the structures of non-derivatized PGE_2 and PGD_2 are also shown. For more details see Section 3.

solid-phase extraction (SPE) [6]. The selectivity of SPE can be greatly enhanced by means of affinity chromatography, especially by immunoaffinity chromatography (IAC) [8–11]. In IAC, the molecule (e.g., PGE_2) to be isolated is specifically and reversibly adsorbed on a complementary binding substance or ligand (e.g., anti-PGE_2 antibody) covalently attached to an insoluble support. IAC is an elegant and highly efficient method to isolate a particular substance from biological samples for analysis by mass spectrometry coupled to GC, CE, or LC. The utility of IAC for the quantitative determination of different prostaglandins including PGE_2 by GC–MS/MS and LC–MS/MS has been demonstrated [12–18].

Usually, experimentally produced antibodies are not specific to a single molecule, but they may bind additional, commonly structurally closely related molecules. This phenomenon is generally known as cross-reactivity. In bioanalytical chemistry, implementation of processes of high specificity (e.g., low cross-reactivity) allows for high analytical performance in terms of accuracy. Yet, the cross-reactivity of antibodies may also be utilized to isolate biomolecules in addition or instead of the biomolecule that binds to a certain antibody with the highest affinity. GC–MS/MS and LC–MS/MS have the greatest potential for utilizing cross-reactivity in analytical processes for low-molecular-mass compounds. Capillary electrophoresis coupled to tandem mass spectrometry (CE–MS/MS), on the other hand, seems to be more suitable for macromolecules (i.e., peptides and proteins).

In previous work, we have demonstrated the excellent suitability of commercially available PGE_2-IAC columns for the quantitative determination of PGE_2 in biological samples by GC–MS/MS [18]. Given the considerable cross-reactivity of about 19% of the commercially available PGE_2-IAC columns toward PGE_1, we investigated whether this feature of PGE_2-IAC columns could be utilized for the specific measurement of PGE_1 in human plasma by GC–MS/MS. Previously, we have been successful in measuring PGE_1 and its metabolites in human plasma by GC–MS/MS after SPE and thin-layer chromatographic (TLC) separation [19]. However, this method requires large plasma volumes (i.e., 5 mL) and yields chromatograms of relatively high noise despite use of GC–MS/MS, which disproves the method's sensitivity in terms of the lower limit of