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Profiling of carbonyl compounds in serum by stable isotope labeling - Double precursor ion scan - Mass spectrometry analysis

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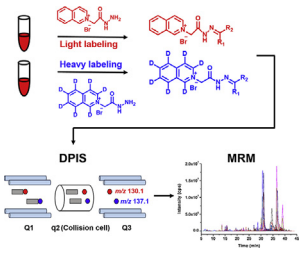
HIGHLIGHTS

- Four labeling reagents were synthesized and compared to label carbonyl compounds.
- IL-LC-DPIS-MS analytical strategy was developed for profiling and quantitation of carbonyl compounds in human serum.
- 156 carbonyl compounds candidates were detected in human serum.
- 44 carbonyl compounds exhibited significant difference between myelogenous leukemia patients and healthy controls.

GRAPHICAL ABSTRACT



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ABSTRACT

Carbonyl compounds are considered as the potential biomarkers for oxidative stress and many types of diseases; therefore their determination may serve as indicator for early clinical diagnosis. Here we developed a strategy based on isotope labeling combined with liquid chromatography-double precursor ion scan-mass spectrometry (IL-LC-DPIS-MS) analysis for comprehensive profiling and relative quantitation of carbonyl compounds in human serum. First, we chose labeling reagents (2-(2-hydrazinyl-2-oxoethyl)isoquinolin-2-ium bromide, HIQB; *N*,*N*,*N*-triethyl-2-hydrazinyl-2-oxoethanaminium bromide, THB; Girard reagent T, GT; Girard reagent P, GP), all of which contain reactive group, isotopically labeled moiety and ionizable group to selectively label carbonyl compounds. Since HIQB labeling offered the best detection sensitivities for carbonyl compounds among these labeling reagents, we used HIQB and the corresponding isotope-labeled reagent of d_7-HIQB as the optimal isotope-labeled reagent. The HIQB and d_7-HIQB labeled carbonyl compounds can generate two characteristic product ions of *m*/*z* 130.1/137.1 under collision-induced dissociation (CID), which contain an isotope tag and therefore were used for double precursor ion scans in mass spectrometry analysis. Using this strategy, 156 carbonyl compounds candidates were detected in human serum, 12 of which were further identified by commercial standards. Subsequently, a targeted method using multiple reaction monitoring (MRM) detection mode was developed for relative quantification of carbonyl compounds in human serum from myelogenous leukemia (ML) patients and healthy controls. As a result, 44 carbonyl compounds were found to have significant difference between ML patients and healthy controls, suggesting that these carbonyl compounds may play certain roles in ML and also can serve as indicators for ML. Taken together, the isotope

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labeling combined with tandem mass spectrometry analysis demonstrated to be a powerful strategy for identification and quantification of carbonyl compounds in serum samples.

1. Introduction

Carbonyl compounds that widely exist in biological systems are generally produced by free-radical-induced reaction with cellular lipid [1,2]. For example, saturated aldehydes such as hexanal, heptanal and nonanal are formed by the peroxidation of ω3 and ω6 fatty acids [3]. As the secondary oxidation products of lipid peroxidation following oxidative stress, carbonyl compounds can exacerbate oxidative damage [4]. Due to the high reactivity, these molecules can act inside and outside the cells, interacting with biomolecules such as nucleic acids and proteins and often damaging the delicate mechanisms involved in cell functionality [5]. In this respect, carbonyl compounds are considered as the potential biomarkers for oxidative stress and various diseases, such as lung cancer [6], alzheimer disease [7], breast cancer [8]. Therefore, they may serve as an important indicator for early clinical diagnosis.

Mass spectrometry (MS) is one of the most prominent platforms for metabolomics study [9,10]. However, the MS response often fluctuates. In this regard, internal standard is normally added to the samples to correct the variation of MS responses. However, many internal standards are expensive and some are not commercially available and difficult to obtain. On the other hand, the determination of carbonyl compounds is cumbersome and easily affected by matrix because carbonyl compounds normally have poor ionization efficiency during MS analysis [6]. To solve these problems, in-vitro isotope labeling strategy has been developed for quantitative analysis of metabolites [11–13]. The ionization efficiencies of target compounds could be enhanced through introduction of permanently charged moieties, or easily protonated moieties in positive mode or easily deprotonated moieties in negative mode [14–17]. In addition, the isotope-labeled analogues were often used as the internal standards to minimize the quantitation deviation due to matrix and ion suppression effects [18].

Neutral loss scan (NLS) and precursor ion scan (PIS) which monitor the characteristic fragment ions produced by the fragmentation of precursor ions are two useful scan modes of triple quadrupole mass spectrometer [19,20]. Compared with full scan mode, NLS and PIS offer better detection sensitivity due to the improved selectivity. Recently, our group successfully developed stable isotope labeling strategy coupled with double neutral loss scan (DNLS) or double precursor ion scan (DPIS) mass spectrometry analysis for the non-targeted profiling of aldehyde- [9], carboxyl- [21], thiol-containing metabolites [14,22] and ribose conjugates [23].

Here, we developed a strategy for non-targeted profiling of carbonyl compounds in human serum by stable isotope labeling combined with liquid chromatography-double precursor ion scan mass spectrometry (IL-LC-DPIS-MS) analysis. In this strategy, we chose and compared four reagents (2-(2-hydrazinyl-2-oxoethyl) isoquinolin-2-ium bromide, HIQB; *N*,*N*,*N*-triethyl-2-hydrazinyl-2-oxoethanaminium bromide, THB; Girard reagent T, GT; Girard reagent P, GP), which contain reactive group, isotopically labeled moiety and ionizable group to selectively label aldehyde and ketone (Fig. 1a). We finally used HIQB and the isotope labeling reagent of d_7-HIQB as the optimal labeling reagent for selective labeling of carbonyl compounds (Fig. 1b). The HIQB and d_7-HIQB labeled compounds can generate two characteristic product ions of *m/z* 130.1 and 137.1, which contain isotope tag and therefore were used for double precursor ion scans in mass spectrometry analysis. In addition, a targeted method was used for relative quantification of carbonyl compounds in serum from myelogenous leukemia (ML) patients and healthy controls.

2. Experimental section

2.1. Chemicals and reagents

Butanal, 2-butanone, pentanal, isovaleraldehyde, hexanal, heptanal, octanal, nonanal, decanal, undecanal, *n*-tetradecanal, benzaldehyde, acetophenone, phenylpropyl aldehyde, vanillin, 17α-hydroxypregnenolone, α-ketoglutaric acid, ethyl bromoacetate, Girard reagent T, Girard reagent P were purchased from J&K Co., Ltd (Beijing, China). Dehydroepiandrosterone, testosterone, pregnenolone, 17-hydroxyprogesterone, ethyl chloracetate were purchased from Energy Chemical Co., Ltd (Shanghai, China). Progesterone and androstenedione were purchased from Aladdin Industrial Co., Ltd (Shanghai, China). D_7-isoquinoline was purchased from Sigma (St. Louis, MO, USA). Analytical grade isoquinoline, triethylamine, ethyl alcohol, hydrazine hydrate, ethyl acetate (EtOAc), *n*-hexane, methyl *tert*-butyl ether (MTBE), sodium chloride, glacial acetic acid (CH_3COOH) were supplied by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). HPLC-grade acetonitrile (ACN) and methanol (MeOH) were obtained from Tedia Co. (Fairfield, OH, USA). Ultrapure water was purified by a Milli-Q apparatus (Millipore, Bedford, MA).

The stock solutions of HIQB (10 μmol/mL), d_7-HIQB (10 μmol/mL), THB (10 μmol/mL), GT (10 μmol/mL) and GP (10 μmol/mL) were prepared in HPLC-grade MeOH. The stock solutions of carbonyl compounds standards were prepared in HPLC-grade MeOH at the concentration of 1 μmol/L for each. All stock solutions were stored at −20 °C.

2.2. Collection of human serum samples

The serum samples from 17 myelogenous leukemia (ML) patients and 17 healthy controls were collected from Taihe Hospital of Hubei University of Medicine (Shiyan, Hubei, China). Detailed information can be found in Table S1 in the Supporting Information. Healthy control serum samples were selected based on medical history and physical examination. The collected serum samples were stored at −80 °C until use. All the experiments were performed in accordance with Taihe Hospital of Hubei University of Medicine Ethics Committee's guidelines and regulations.

2.3. Synthesis of labeling reagents

Chemical labeling is primarily used to improve ionization efficiency during MS analysis. Chemical labeling strategy is then aimed at the incorporation of a group with a permanent charge or other groups that enhance ionization efficiency [11]. In addition, chemical labeling may also improve the fragmentation behavior in tandem MS analysis [2,11]. In this respect, we employed the compounds of HIQB, THB, GT and GP to label carbonyl compounds.